4162

THE JOURNAL OF HORTICULTURAL SCIENCE

(Formerly The Journal of Pomology and Horticultural Science)

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Vol. XXVI

1951

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PRINTED AND MADE IN GREAT BRITAIN BY HEADLEY BROTHERS LTD ASHFORD KENT

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EFFECTS OF ETHYLENE UPON RESPIRATORY ACTIVITY OF APPLES IN GAS STORAGE, WITH SPECIAL REFERENCE TO STAGE OF MATURITY

By **DOROTHY G. GRIFFITHS** and **NINA A. POTTER**

Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research

In previous work (Griffiths and Potter, 1949) a comparison was made of two different methods of storage at 5° C. using King Edward VII apples ; (A) in which the products of respiratory activity were allowed to accumulate in the container until the desired concentration of $CO_2 + O_2$ viz. 8% CO_2 + 13% O_2 + 79% N_2 was reached, that concentration being then maintained by controlled ventilation ; and (B) in which the fruit was held in a container through which was passed a synthetic gas mixture composed of 8% CO_2 + 13% O_2 + 79 % N_2 at a controlled rate not allowing either the CO_2 of respiration or the volatiles to accumulate. The concentrations of ethylene and odorous volatile substances observed, expressed as mg. C per 100 litres of gas, were as follows :

Method	(A)	Ethylene 12-25 mg. C ;	Odorous	2-3 mg. C.
,,	(B)	,, 1-2 mg. C ;	,,	1-2 mg. C.

and the weight of apples stored under conditions (A) and (B) were 127 kg. and 4 kg. respectively.

The respiratory activity of the fruit was depressed at first in both cases, but after the first 10 days that of sample (A) rose to a level considerably higher than that of (B), and this difference was thereafter maintained (Fig. 1).

In the following year the experiments described in the present paper were carried out to determine whether the higher maintained level of respiratory activity of the apples stored under the conditions of Method A could be obtained by giving specific treatment with ethylene to apples stored under conditions (B). Accordingly, three concentrations of ethylene were added to the synthetic gas mixture in which King Edward VII apples at three different stages of maturity were stored under conditions (B), which allowed neither volatile substances nor CO_2 to accumulate.

The respiration of four samples from each picking was determined in the gas mixture, and three of these samples were treated with different concentrations of ethylene after the apples had attained a steady rate of CO_2-production. The fourth was used as a control without ethylene treatment.

The three concentrations of ethylene used were : (1) 0·05 %, (2) 0·01%, and (3) 0·002%. The respiration of the fifth sample from each gathering was determined in air.

The samples of fruit, each weighing 4 kg., were stored in large desiccators of 13 litres capacity, which were ventilated at a rate sufficient to prevent the respiratory CO_2 rising to more than 0·9 per cent.

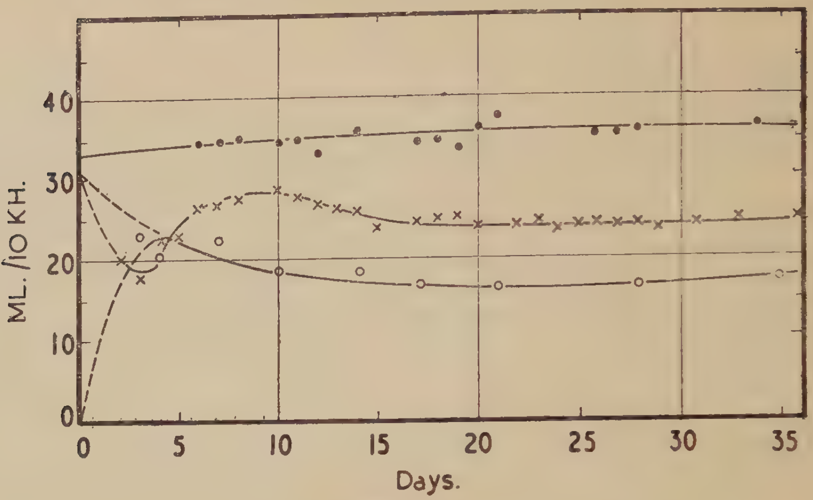

FIG. 1.

Production of CO_2 by King Edward VII apples stored at 5°C.

●—● In air in desiccator.

×—× In cabinets with accumulation of volatile substances and CO_2 until the concentration of $CO_2=8\%$.

○—○ In a synthetic mixture of $8\% CO_2 + 13\% O_2 + 79\% N_2$ in desiccator.
Gathered 23·10·47.

EXPERIMENTAL.

The first gathering was made on 12th August, 1948, when the apples were immature, hard and green, their average weight being 83·4 grams. Five comparable samples, each weighing approximately 4 kg. and containing 48 apples, were selected for use in the first experiment.

For the second experiment the apples were picked on 20th September, 1948. They were immature, hard and green, and their average weight was 129 grams. Each of the five samples weighed approximately 4 kg. and contained 31 apples. These two experiments were started as soon as the fruit was in the laboratory immediately after gathering.

The third gathering was made at the time of the commercial picking on 18th October, 1948. The fruit was still fairly hard and green. The average weight of the apples was 143 gm., and each of the five samples contained 28 apples and weighed approximately 4 kg. These samples were kept at 5° C. in air until the third experiment was started on 25th October, 1948.

Samples 1, 2 and 3 in each of the Experiments I-III were treated with 0·05, 0·01 and 0·002 per cent. ethylene respectively after they had been in the gas mixture for 12-14 days.

The gas mixture, which was contained in a cylinder fitted with a needle valve and a mercury regulating system, was humidified by bubbling through a bottle containing dilute H_2SO_4, which was connected to the desiccator by means of a T piece, one arm of which could be attached to the ethylene container when required. The gas then flowed through the desiccator into a gas sampling tube fitted with a by-pass, so that the gas stream was not interrupted when the tube was removed for analysis of the gas. The gas stream was then dried over calcium chloride before passing through the flowmeter in order to prevent condensation in the capillary of the meter.

The apparatus for the ethylene treatment was of a simple type and consisted of two aspirators, each of approximately 11 litres capacity, which were arranged one above the other, the upper one being a Marriott's bottle, which was filled with a known volume of water saturated with ethylene at 5° C.

The lower aspirator contained a mixture of ethylene and air in the correct proportions for producing the required concentration of ethylene in the gaseous stream. The upper aspirator was connected to the lower aspirator by a fine capillary tube, so that water flowed at a definite rate from the top jar and displaced the required volume of ethylene-air mixture, which was bubbled through a small trap containing 0·5 ml. of water to prevent back diffusion, to join the main gas stream through the T piece attached to the desiccator.

The respiratory activity of the fruit in air (Sample 5) was determined in a similar apparatus, but compressed air from a cylinder was used in place of the gas mixture and was humidified by bubbling through 8 per cent. KOH solution.

The respiratory activity of the five samples in each gathering was determined at the same time in a constant-temperature room at 5° C., but it was found that even when all the desiccators and aspirators were completely covered with several layers of insulating material, there were slight temperature differences, not exceeding 1° C., between the samples. Duplicate analyses of the gas stream emerging from the desiccators were made daily and determinations of the gas mixture in the cylinders were made at frequent intervals.

The rate of flow was maintained at approximately two litres per hour throughout the experiments, consequently there was an interval at the beginning of each experiment before the air in the desiccator was completely replaced by the cylinder gas. This interval was determined experimentally by using a desiccator without the apples but containing an equivalent air space, and by frequently analysing the gaseous mixture. The interval was found to be 48 hours.

RESULTS.

The rate of production of CO_2 by the apples is calculated from the results of analysis of the gas samples and the flow-meter readings, and is expressed as ml. of CO_2 per 10 kg. per hour.

The effect of ethylene, even at the highest concentration used (0·05 per cent.), was slight. No obvious difference could be observed between the effects of the lowest and of the highest dosages. The results from the four samples in the gas mixture are therefore averaged and the curves are smoothed by eye judgment. They are shown in Fig. 2. In the first gathering, the first three points of the respiration in air are doubtful and they are so treated in the smoothed curve.

During the first few days in gas mixture, the sap of the fruit is absorbing CO_2, so that the curves should show a zero or negative respiratory activity at zero time, which rises to its true value in approximately 4 days. The amount of CO_2 absorbed has not been brought into account, but calculation indicates that it would correspond approximately to the amount required if the true respiratory activity from the outset were as shown by extrapolation to zero time of the respiratory activity line established after the first four days.

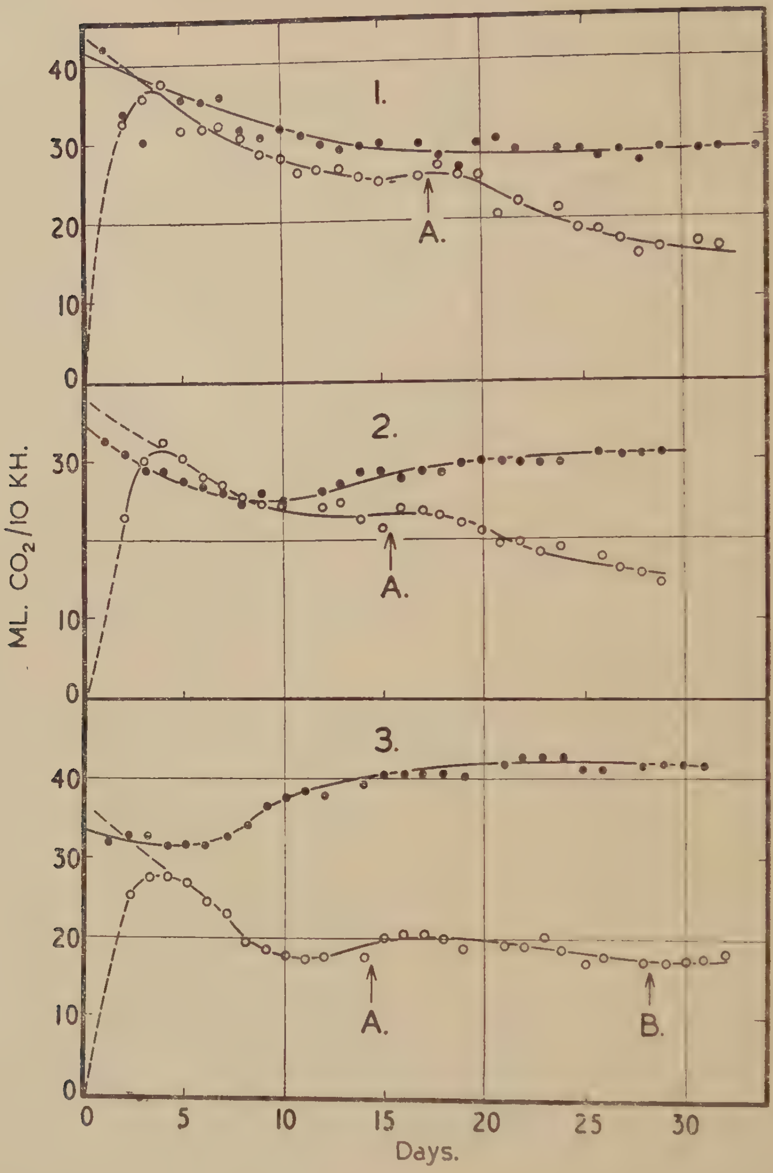

FIG. 2.

Respiratory activity of King Edward VII apples stored at 5°C. at 3 stages of maturity.

●—● CO_2 production by 1 sample of apples in air.

○—○ Mean CO_2—production of 4 replicate samples of apples in synthetic mixture of $8\% CO_2 + 13\% O_2 + 79\% N_2$.

A=beginning of ethylene treatment in 3 samples.
B=ethylene treatment ended.
Gathered (1) 12.8.48, (2) 20.9.48, (3) 18.10.48.

DISCUSSION.

The effect of ethylene treatment, in concentrations from 20 to 500 parts per million, on the respiratory activity after the apples had been in the synthetic gas mixture for 15-20 days was slight. The stage in the storage life at which ethylene treatment was given must be borne in mind. It has already been reported (Kidd and West, 1937b) that the respiratory activity of Bramley's Seedling apples can be stimulated without recovery by treatment with 1,000 parts per million of ethylene at a later stage in the storage life in an atmosphere containing 10 per cent. CO_2 and 15-20 per cent. O_2. The method these authors employed, however, is thought to be more like Method A than Method B, as regards the removal from the fruit of ethylene produced by the fruit.

Before the samples of fruit were treated with ethylene a surprisingly large variation in the respiratory activity of the four samples in the gas mixture was noticeable. This variation is greater than that found with samples of similar size in air. Table I shows the range of these differences, which could not be traced to any systematic error connected with the method of experimentation. In the same Table are given comparable results, kindly provided by Dr. A. C. Hulme, with Bramley's Seedling apples in air. It is therefore tentatively accepted as a fact that the variation in respiratory activity between individual fruits in these experiments was, at their particular stage of ontogeny, greater in the synthetic gas mixture than it was in air.

Previous experimental work (Kidd and West, 1933 and 1937a) on the effects of CO_2 upon the respiratory activity of apples at different stages of maturity and senescence has shown that in an early stage of growth the effect is that of considerable depression of the respiratory activity, established within 20 days. In the six weeks before the climacteric rise, on the other hand, the effect of CO_2 is to increase the respiratory activity. After the climacteric rise the effect of CO_2 is always that of a depressant. In the light of these considerations it may be suggested that at the stage of ontogeny of the apples used in the present experiments, there was individual variation not only in respiratory activity but also in the effect of the synthetic gas mixture upon the fruits, the respiratory activity of some individuals being decreased and that of others increased. This explanation of the variation observed is put forward tentatively, and it is emphasized that further study is required of the respiratory activity of apples under the influence of gas mixtures containing carbon dioxide.

The differences observed in the respiratory activity of the two samples stored under conditions (A) and (B) (Fig. 1) may have been due to the fact that the 4 kg. sample used in the latter case was not large enough to ensure that the results were approximately average ones for the population as a whole.

There are however certain further features in our present and previous experiments which incline us to prefer a different interpretation. Thus, in the present experiment, while the difference in the respiratory activity of the four samples was large at first, it steadily diminished, suggesting that individual fruits, with advance in age, are tending to react in a more uniform manner to the influence of the gas mixture. Again, the trends of the respiratory activity of the two earlier gatherings are steadily downwards. They do not show the sharp decline at first (15-20 days) due to a depressant effect of CO_2 followed by a slow downward drift. In the previous year's experiment (Fig. 1) the main depressant effect of the gas mixture was established in 10-15 days. The apples were also more advanced in maturity and were well on the climacteric rise before storage, compared with those of the third gathering of the present experiment which were definitely pre-climacteric. The interpretation, therefore, of the results shown in Fig. 1 which appears to us most likely to be correct, is as follows: In the case of the small sample of apples held under conditions (B), the fruit had already reached the climacteric rise in respiratory activity when brought under the influence of the gas mixture. The continuous removal of the ethylene which is formed and the presence of CO_2 lead to recovery from ethylene stimulus, and the fruit then shows the respiratory activity typical of depressed pre-climacteric fruit. In the case of the large sample stored under conditions (A), the accumulation of ethylene up to 200 parts per million in the first few days leads to the completion of the climacteric in spite of the presence of 8 per cent. CO_2, and the respiratory activity is subsequently that of depressed post-climacteric fruit.

TABLE I.

Range of the respiratory activity of four replicate (4 kg.) samples of King Edward VII apples in 8% CO_2 *+ 13%* O_2 *+ 79%* N_2 *expressed as percentage of the mean.*

Gathered 12/8/48.		Gathered 20/9/48.		Gathered 18/10/48.	
Days in store.	%	Days in store.	%	Days in store.	%
3–5	22·0	3–5	11·8	2–4	16·6
6–8	14·0	6–8	14·1	5–7	21·7
9–11	12·6	9–12	13·6	8–10	21·6
12–14	9·0	13–15	8·3	11–14	21·8
				28–32	13·3

Range of respiratory activity of replicate (3 kg.) samples of Bramley's Seedling apples in air expressed as percentage of the mean.

Gathered.	No. of samples.	%
3/8/37 (pre-climacteric)	11	5·1
15/9/38 „	4	2·8
15/9/39 „	5	1·7
4/10/39 „	5	1·4
5/10/49 (on the climacteric)	4	12·9
25/10/49 (post-climacteric)	3	2·1

SUMMARY.

King Edward VII apples were stored in an atmosphere containing 8% CO_2 + 13% O_2 + 79% N_2, which was continually renewed at a rapid rate from a cylinder containing the synthetic gas mixture. The continuous addition of ethylene in concentrations between 20 and 500 parts per million had little effect upon the respiratory activity when the apples were stored at 5° C. in the pre-climacteric state (gathered between 12th August and 18th October) and when the ethylene treatment was given in the first five weeks. The degree of variation in respiratory activity

between 4 kg. samples in the artificial gas mixtures was much larger than the variation to be expected from our previous observations of respiratory activity in air. An explanation for this is suggested, and the bearing of the above observations on results previously reported is discussed.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research, and is published by permission of the Director.

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(*Received* 16/11/49.)

" HARD-CORE "—A NUTRITIONAL DISORDER OF TOMATOES

By ELSA B. KIDSON*
Cawthron Institute, Nelson, New Zealand

OUTDOOR tomatoes in the Nelson district are subject to a form of abnormal ripening known locally as " hard-core ". This disorder appears to be increasing in severity and becoming more widespread. The indications are that it is caused by some nutritional disturbance and is influenced to some extent by seasonal conditions. Field experiments and analytical work carried out during recent years at the Cawthron Institute have provided a considerable amount of information, and though it cannot be claimed that all the factors influencing the occurrence of hard-core have been identified, certain interesting results have been obtained which are here recorded.

DESCRIPTION OF THE DISORDER.

The fruits of affected plants are characterized by a part of the tissue which remains greenish as the rest of the fruit ripens. The abnormal tissue occurs at the stem end and varies in extent with the severity of the disorder. In very badly affected fruit the hard-core may extend through nearly all the solid tissue of the tomato, though the seed-sacs appear normal. The disease may or may not be accompanied by " yellow-back " on the outside of the tomato at the stem-end, and in severe cases there is sometimes a general patchy yellow appearance of the flesh visible through the skin. The hard-core tissue is tough, even when the tomato is over-ripe, and fruit affected by the disorder is unsuitable for canning. (FIG. I.)

Plants susceptible to severe hard-core tend to develop a stunted appearance as the season progresses, with rather weak, light-coloured foliage towards the top of the plant. The lower leaves, though apparently healthy in the early stages, often develop a purpling and necrosis by mid-season. The crop may be comparatively heavy. In the Kondine variety, bad hard-core has been found on plants producing 7 lb. of fruit with the normal fertilizer programme and also on plants with no fertilizer and producing as little as 3·8 lb. of fruit per plant. Badly affected plants produce hard-core fruit throughout the season, though the severity diminishes considerably, late in the season, when ripening is slow. Though the amount of hard-core may vary from plant to plant it is usual for all plants on an area subject to bad hard-core to show symptoms of the disorder.

TOMATO VARIETIES AND SUSCEPTIBILITY TO " HARD-CORE ".

Severe hard-core has been found on plants of the following varieties : Kondine, Blakeley, Blenheim, Exhibition, Potentate, Halls, and Australian Dwarf Red. The disorder appears to be worse in varieties producing a large amount of fruit and it is likely that other heavy-cropping varieties may be equally susceptible. Bonny Best and Marglobe, which in the Cawthron Institute experimental field give

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* Seconded by the Department of Scientific and Industrial Research.

PLATE I.

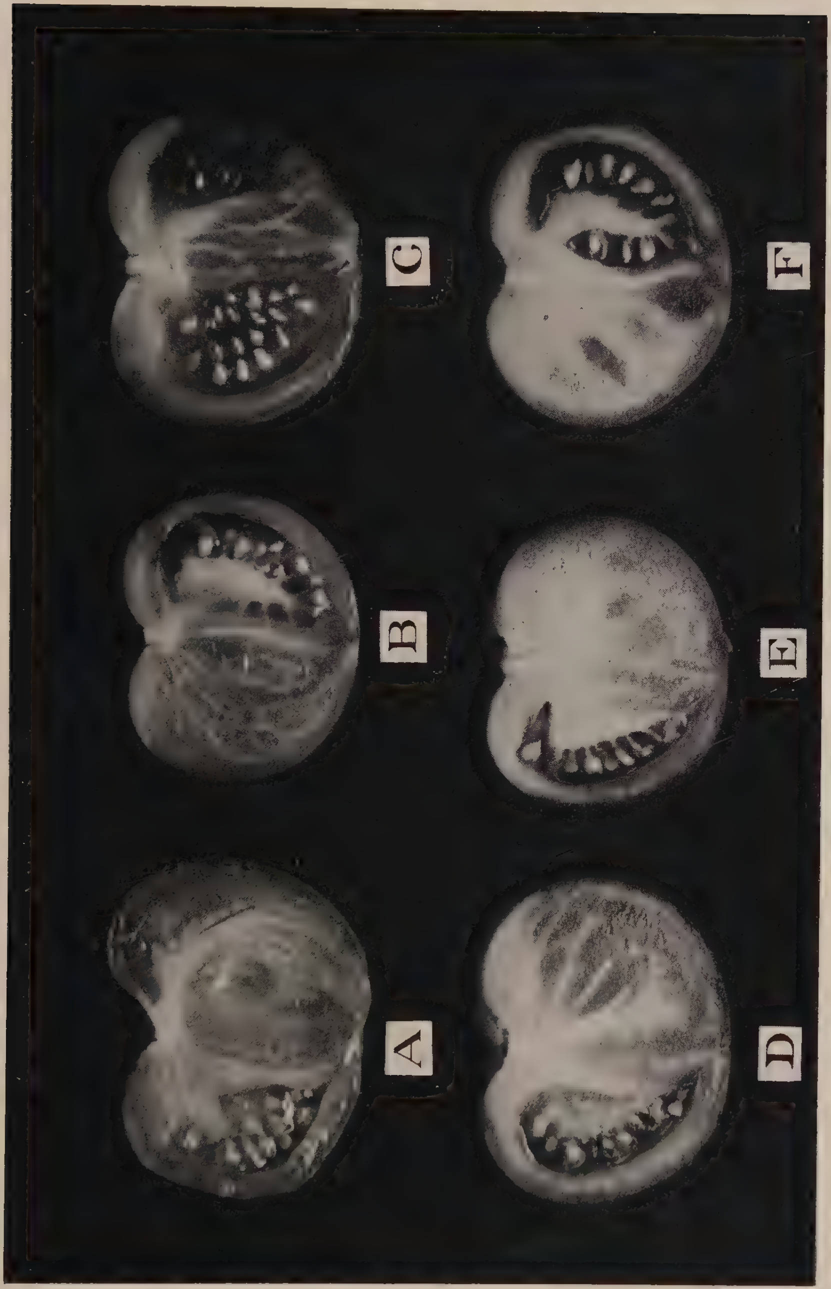

FIG. 1.

“Hard-Core” in Tomatoes, Kondine variety

A. No hard-core. B. and C. Slight hard-core. D., E. and F. Bad hard-core.

a much smaller yield of fruit, do not appear to be severely affected. In the variety Kidson, a heavy-cropper not much subject to the disorder, there is often a hollowness and abnormal softness of the fruit which may be a related symptom. Whereas some varieties, e.g. Kondine, usually show yellow-back when the disorder is severe, others, particularly certain small-fruited ones, may show no external symptoms, the hard-core tissue being completely covered by a thin red layer under the skin.

CHEMICAL COMPOSITION OF THE SOIL IN RELATION TO THE INCIDENCE OF "HARD-CORE".

It has been found in the Cawthron tomato field that although under standard fertilizer practice the whole area produces hard-core, the severity of the disorder varies from place to place, certain areas being less affected than others year after year. This variation in the amount of hard-core is associated with variations in the chemical properties of the soil.

In Table I are given chemical analyses of the soils of certain experimental blocks on the tomato field, varying in their susceptibility to hard-core.

Figures for exchangeable bases and total nitrogen are expressed on the oven-dry basis. The leaching solution used for the determination of exchangeable bases was normal ammonium acetate at pH7.

TABLE I.

The relation of the chemical composition of the soil to the incidence of "Hard-core".

Block.	pH.	Exchangeable bases in milli-equivalents (*m.e.*) per cent.			Total Nitrogen per cent.	Amount of "Hard-core".
		Ca	Mg	K		
L	7·9	20·1	7·3	0·21	0·20	Bad.
E	6·9	11·2	7·8	0·11	0·14	Bad.
A	7·6	18·8	8·7	0·35	0·18	Bad.
K	6·9	20·8	4·1	0·30	—	Bad.
G	8·2	20·2	6·8	0·73	0·23	Medium to fairly bad.
C	7·2	24·6	3·8	0·80	0·20	Medium.
G(1)	8·0	31·0	4·6	1·88	0·22	Slight.

The most outstanding feature of these analyses (Table I) is the inverse relationship between the amount of exchangeable potassium in the soil and the severity of the hard-core produced on it. The exchangeable potassium varies from 0·11 m.e. per cent. to 1·88 m.e. per cent. Badly affected soils have less than 0·40 m.e. per cent. Only slight hard-core occurs on the soil with 1·88 m.e. and moderate hard-core on soils with 0·73 m.e. and 0·80 m.e. per cent. of exchangeable potassium. No such consistent relationship is found for exchangeable calcium or magnesium or total nitrogen, though there is a tendency for total nitrogen to be slightly lower with bad hard-core than with medium or slight hard-core.

Analysis of samples from other parts of the Nelson district has given indications of a similar association of low hard-core with high exchangeable potassium and vice versa in any particular soil type. On different soil types, however, the same figure for exchangeable potassium may be associated with very different susceptibility to the disorder. Thus with 0·20 m.e. per cent. of exchangeable potassium, a granite soil produced only slight hard-core, whereas on the heavier soil of the Cawthron Institute grounds with the same percentage of exchangeable potassium the plants were very badly affected.

High magnesium content was found in three of the four Cawthron soils most susceptible to hard-core. In other parts of the Nelson district plants have shown much hard-core on soils of quite low magnesium content.

Determinations of pH values on the Cawthron Institute experimental plots and in other tomato soils in the Nelson district have given no indication that hard-core is associated with any particular degree of soil acidity. Thus bad hard-core has been found on soils with pH values between 6 and 8, and slight hard-core between 5·5 and 8.

So far no chemical characteristic of the soil except exchangeable potassium has been found to correlate with susceptibility to hard-core.

Chemical composition of the plant in relation to the amount of " Hard-core ".

Analyses of tomato fruits and leaves from plants showing varying degrees of hard-core are given in Tables II and III.

Table II.

Relation of potassium and nitrogen content of tomato fruit to susceptibility to " Hard-core ".

Sample No.	Variety.	Amount of " Hard-core ".	K_2O	N
			per cent.	per cent.
1167 . .	Kondine	None	4·9	—
H.C.16 . .	,,	Very slight	4·9	1·84
H.C.14 . .	,,	Medium	3·5	1·70
1140 . .	,,	Fairly bad	3·7	—
H.C.12 . .	,,	Bad	3·2	1·85
H.C.20 . .	Australian Dwarf Red	None	6·4	3·06
H.C.22 . .	,,	Slight to Medium	4·7	2·92
H.C.19 . .	,,	Very bad	2·8	2·34
H.C.1 . .	Halls	Slight	4·2	2·14
H.C.6 . .	,,	Slight to Medium	4·8	2·25
H.C.3 . .	,,	Medium to fairly bad	4·6	2·19
H.C.4 . .	,,	Fairly bad	4·2	2·37
H.C.7 . .	,,	Bad	3·0	2·30
H.C.23 . .	Exhibition	Slight	4·2	1·45
H.C.18 . .	,,	Very bad	2·7	1·84

Percentages are on the dry-matter basis.

TABLE III.

Relation of the chemical composition of the leaves to the amount of "Hard-core".

Sample.	Variety.	Position of leaf from bottom of plant.	"Hard-core."	CaO per cent.	MgO per cent.	K_2O per cent.	N per cent.	Na per cent.	P_2O_5 per cent.	SO_3 per cent.	Cl per cent.
1139	Kondine	12th	Fairly bad	8·48	3·54	1·38	—	0·06	0·84	3·18	0·30
1141	,,	,,	None	—	—	4·23	—	0·05	—	5·35	0·15
1143	,,	,,	None	8·94	1·13	3·73	—	0·20	0·67	4·50	1·02
1270	,,	15th and 16th	Slight	8·75	1·98	2·34	3·33	—	1·09	—	—
1290	,,	,,	Bad	8·63	2·24	0·71	3·33	—	0·83	—	—
1584	,,	14th and 15th	Medium	10·31	2·47	2·01	—	—	—	—	—
1588	,,	,,	Bad	9·38	2·82	1·38	—	—	—	—	—
1172	Blenheim	12th	Bad	9·31	—	0·81	—	0·20	—	1·97	0·51
1171	,,	,,	Slight	8·62	1·62	4·54	—	0·04	0·63	2·98	0·42
1169	,,	,,	Very slight	—	—	2·40	—	0·09	—	7·05	0·58

Percentages are on the dry-matter basis.
Samples included the leaflets only. The petioles were discarded.

Fruit.

Analyses have shown that there is a tendency for fruit with hard-core to be low in potassium, and for the amount of hard-core to be inversely related to the amount of potassium (Table II). Thus in the Australian Dwarf Red variety, healthy fruit contained 6·4 per cent., fruit with slight to medium hard-core 4·7 per cent., and badly affected fruit 2·8 per cent. K_2O on the dry matter basis. Similar tendencies are apparent for other varieties. It will be seen from Table II that the amount of nitrogen in the fruit, on the dry-matter basis, does not appear to be related to the amount of hard-core. A limited number of analyses in addition to those reported in Table I, showed no connection between the content of Ca, Mg, P, S, Mn, B or Fe in the fruit and the degree of hard-core.

Leaves.

From Table III it will be seen that in the leaves, as in the fruit, severe hard-core is associated with a low potassium content. Thus, the samples from badly affected plants had less than 1·4 per cent. K_2O, while those from healthy plants had more than 3·7 per cent. There is also an indication that magnesium may be high where hard-core is bad. From these and other analyses no consistent relationship has been found between the severity of the disease and the leaf content of Ca, N, Na, P, S, Cl, Mn, B or Fe.

EFFECT OF SOIL TREATMENTS ON THE INCIDENCE OF "HARD-CORE".

During the course of the past seven seasons, many soil treatments have been tried in an attempt to eliminate the disorder. Certain of these treatments affected the amount of hard-core produced, some having a beneficial effect on fruit quality and some increasing the severity of the disorder. The data and discussion following are mostly limited to these treatments.

The field experiments were located in blocks L, A, K and C (Table I) or on similar blocks. All had a high phosphate status owing to previous fertilizer treatment. In most cases the variety Kondine was grown as being very susceptible to hard-core, though Potentate was used for some experiments. The plants were staked 15 inches apart with 3 feet between the rows. The normal yield was 5 to 7 lb. per plant. The estimate of the effect of treatment on the amount of hard-core was based on an examination of the whole crop in most seasons, and of one or more pickings at the height of the season in other years. To determine the amount of hard-core all fruit was cut as illustrated in the photograph. It was recorded in a number of grades. The hard-core was classified as "commercial" when about one-third or more of the surface of the cut fruit showed hard-core tissue.

1. *Effect of Fertilizers.*

The effect of certain fertilizer treatments on the susceptibility of the plants to hard-core is discussed below. The figures given in the tables are the averages for a number of replicated plots except where otherwise specified.

It will be seen from Table IV that hard-core was worse on plots without fertilizer. The average percentage of badly affected fruit from the no-fertilizer plots was 35, whereas the figure for the standard fertilizer plots was 24. Omitting potash or nitrogen from the standard fertilizer appears to have caused a slight increase in hard-core in some seasons, but the detrimental effect of these treatments was not as marked

TABLE IV.

Effect of fertilizer treatment on the amount of "Hard-core" in tomato plants.
(Block L.)

Treatment.	Percentage of badly affected fruits.						Percentage of healthy and slightly affected fruits.					
	1942-43.	1943-44.	1944-45.	1945-46.	1946-47.	Average.	1942-43.	1943-44.	1944-45.	1945-46.	1946-47.	Average.
No fertilizer ..	34	16	24	26	75	35	34	9	20	12	2	15
Standard fertilizer ..	18	6	25	17	52	24	45	25	18	16	7	22
Standard minus potash ..	18	13	20	22	65	28	51	25	16	10	6	22
Standard minus nitrogen ..	27	9	13	18	65	26	35	28	23	16	5	21
Standard plus ½ ton K_2SO_4 per acre ..	10	2	17	1	21	10	60	50	23	53	17	41
Standard plus ½ ton KCl per acre ..	13	3	22	2	22	12	60	52	19	47	18	39

Results are expressed as number of fruits per cent.

The Standard fertilizer treatment was as follows :

	Base-dressing.	*Top-dressing.*
Superphosphate	300 lb. per acre	200 lb. per acre.
Sulphate of potash	120 lb. ,,	80 lb. ,,
Sulphate of ammonia	90 lb. ,,	—
Nitrate of soda	—	60 lb. ,,
Dried blood	90 lb. ,,	60 lb. ,,
Total	600 lb. per acre.	400 lb. per acre.

The base-dressing was applied in trenches along the rows before planting and the top-dressing along the rows in December.

as with the no-fertilizer treatment. Both the no-fertilizer and the no-nitrogen plots produced acute nitrogen-deficiency symptoms including very stunted growth and reduced crop, but in the no-fertilizer plots the percentage of badly affected fruit was higher.

Extra potash applied at the rate of ½ ton of sulphate or muriate per acre along the rows before planting had a beneficial effect on fruit quality. Averages over the five seasons show 10 and 12 per cent. of badly affected fruit for these two treatments (Standard 24 per cent.) and 41 and 39 per cent. of healthy or slightly affected fruit (Standard 22 per cent.). In certain seasons the effect was more noticeable, e.g. the 1945-46 season where approximately 50 per cent. of the fruit was healthy or slightly affected, compared with 16 per cent. for the standard treatment. In the 1946-47 season when fruit quality tended to be very bad throughout, plots with the extra potash treatment had a relatively large percentage of hard-core but nevertheless a considerably smaller amount of badly affected fruit than other treatments. Extra potash gave the least improvement in 1944-45. It may be pointed out that during this season the rainfall was exceptionally high during December, January and February, conditions being unfavourable for tomatoes. Although hard-core was reduced by the application of an extra ½ ton per acre of potash, the treatment did not by any means eliminate the disorder. Analysis indicated that the exchangeable potassium in the soil of the extra potash plots had not been greatly increased by the treatment over the five seasons. A sample taken along the rows after the experiment had been discontinued had an exchangeable potassium level of 0·73 m.e. per cent. compared with 0·22 m.e. for the no-fertilizer treatment. Leaf analysis during the first two years gave little indication of an increased potassium intake on the extra potash plots, but in the 1945-46 season tissue tests showed an increased potassium status, particularly of the older leaves.

TABLE V.

Effect of heavy applications of lime, sulphur and potash on incidence of "Hard-core".

(Block L. Season 1948-49.)

Treatment.	Commercial "Hard-core" per cent.	Range per cent.
Control	42	24–57
2 tons slaked lime	30	16–47
2 tons sulphur	46	34–68
1 ton sulphate of potash ..	12	9–15

Note.—All the plots had standard fertilizer. The lime and sulphur and half the potash were applied broadcast when the soil was dug before planting. The remaining potash was applied in three equal top-dressings along the rows during the season.
Fruit was examined on 9th February and 10th March, 1949.
Results are expressed as number of fruits per cent.

Results of further fertilizer experiments on Block L are reported in Table V. Extra potash fertilizers were applied to one series of plots at the increased rate of

1 ton per acre. The effect of dressings of slaked lime or sulphur at the rate of 2 tons per acre is also shown. The standard fertilizer in the 1948-49 season was double the amount used in previous experiments on Block L.

The beneficial effect of potash is again shown (Table V). Where extra sulphate of potash at the rate of 1 ton per acre was applied, only 12 per cent. of commercial hard-core was recorded, compared with 42 per cent. for the standard treatment. Two tons of slaked lime per acre appear to have brought about some improvement, the fruit showing 30 per cent. commercial hard-core. This may be the result of an increase in the availability of reserves of plant food in the soil as a result of the lime treatment. The lime increased the pH from 6·6 to 7·3, whereas the sulphur decreased it to 5·6. Sulphur appeared to have, if anything, a slightly adverse effect on fruit quality.

2. *Effect of steam and soil disinfectants.*

Soil sterilization with steam has been shown by Rigg *et al.* (1944) to decrease hard-core. Later experiments have proved that other sterilizing agents, particularly chloropicrin, are also beneficial. Data from recent experiments are given below in Table VI.

TABLE VI.

Effects of steam and soil disinfectants on " Hard-core ".
(Blocks A and C.)

Treatment.	Block A.		Block C.	
	No " Hard-core ".	Commercial " Hard-core ".	No " Hard-core ".	Commercial " Hard-core ".
	per cent.	per cent.	per cent.	per cent.
Control	10	61	43	14
Chloropicrin	28	37	80	4
D-D	—	—	66	6

Fruit was examined on 9th February, 1949.

(Block D.)

Treatment.	Commercial " Hard-core ".	
	1945-46.	1946-47.
	per cent.	per cent.
Control	67	76
Steam	32	65*
Chloropicrin	15	29
Formalin 2 per cent. ..	43	74
D-D	—	64

Fruit was examined throughout the season.
Results are expressed as number of fruits per cent.
* Wet soil conditions unfavourable for steam treatment.

Chloropicrin was applied at the rate of 4 ml. per sq. foot to a depth of 6 in. by means of an injector. The soil surface was then moistened and covered with proofed paper for a few days.

Formalin treatment was carried out in two applications each of 2½ gallons of a 2 per cent. solution per sq. yd.

D-D, a proprietary product containing equal parts of dichloropropane and dichloropropylene, was used at the rate of 4 ml. per sq. foot to a depth of 6 in.

On an area such as Block C, sterilization was found to reduce hard-core to a negligible amount but in Block A, which normally produces much worse hard-core, though the improvement with sterilization was marked, the percentage of normal fruit was still relatively small.

Thus from Table VI it will be seen that on Block C, chloropicrin treatment resulted in 80 per cent. of fruit free from the disorder, while on Block A the healthy fruit on the chloropicrin-treated plots was only 28 per cent. of the total. The differences in susceptibility to the disorder between the two areas will be seen from the fact that untreated plots on Block C had 43 per cent. of healthy fruit and the untreated plot on Block A only 10 per cent.

On Block D (Table VI), figures for the 1945-46 season support the previous evidence of the effectiveness of both steam and chloropicrin in reducing the severity of hard-core. In the 1946-47 season chloropicrin again gave a very marked improvement in fruit quality, figures for commercial hard-core on the control and chloropicrin-treated plots being 76 and 29 per cent. respectively. In this season the steamed plots were disappointing, probably owing to the fact that soil conditions were unfavourable for steaming at the time this treatment was applied. Results with D-D indicate that D-D as used in these experiments reduced hard-core but was not as effective as chloropicrin. The same may be said for 2 per cent. formalin. Experiments have shown that chloropicrin owes little or none of its beneficial effect to its nitrogen content as a source of nitrogen for the plant.

3. *Effect of organic manures.*

On Block A plots were set out in the winter of 1945 to determine the effect of liberal dressings of certain organic manures on the incidence of hard-core. The manures were spread over the surface of the plot and dug into the top nine inches. Observations of hard-core on these plots, continued over a number of years, are reported in Table VII.

Cocoa husks and sheep manure produced a startling reduction in hard-core in the seasons immediately following their application (Table VII). Thus in plot 6 where the equivalent of 26·5 tons per acre of dry cocoa husks were applied in the winter of 1945, the commercial hard-core figure in the 1945-46 season was as low as 23 per cent., compared with 76, 82, 67 and 95 per cent. in the plots receiving standard fertilizer. Where the standard fertilizer was used as well (Plot 12) the commercial hard-core was only 5 per cent. Sheep manure at the rate of 18·8 tons of dry matter per acre on plot 15 was also very effective, the plot producing only 13 per cent. of commercial hard-core in the following season. Additional evidence of the beneficial effect of sheep manure was obtained on plot 5. In the 1945-46 season this plot had 95 per cent. of commercial hard-core. In the winter of 1946 sheep manure equivalent to 30 tons of dry matter per acre was applied. For the

TABLE VII.

Effects of rich organic manures and of increased fertilizer applications on the amount of commercial "Hard-core".

(*Block A.*)

Plot.	Treatment.	1945-46.	1946-47.	1947-48.*	1948-49.*
		per cent.	per cent.	per cent.	per cent.
	No Sterilization of the Soil :				
7	Standard fertilizer ..	76	87	96	61
12	Cocoa husks 26·5 tons dry matter per acre 1945, 3·5 tons 1946, standard fertilizer 1945, 1946, 1947, 1948 ..	5	26	48	64
15	Sheep manure 18·8 tons dry matter per acre 1945, 11·2 tons 1946, standard fertilizer 1946, 1947, 1948	13	23	78	56
11	No fertilizer 1945, treble standard 1946, standard plus double nitrogen and double potash 1947 and 1948 ..	80	34	64	8
	Sterilization with Chloropicrin 1948 only :				Sterilized.
2	Standard fertilizer ..	82	71	98	41
13	Standard fertilizer ..	67	81	98	32
6	Cocoa husks 26·5 tons dry matter per acre 1945, 3·5 tons 1946, standard fertilizer 1947 and 1948 ..	23	40	77	14
5	Standard fertilizer 1945, sheep manure 30 tons dry matter per acre 1946, standard fertilizer 1947 and 1948 ..	95	16	55	20
14	No fertilizer 1945, treble standard 1946, standard plus double nitrogen and double potash 1947 and 1948 ..	92	62	99	13

Results are expressed as number of fruits per cent.

**Note.*—Figures for 1945-46 and 1946-47 based on records for the whole season.
Figures for 1947-48 and 1948-49 based on one picking at the height of the season.
For the standard fertilizer see footnote, Table IV. These quantities were used in the first two seasons and doubled for 1947-48 and 1948-49.

next tomato crop only 16 per cent. of commercial hard-core was recorded, whereas the controls had 71 and 87 per cent.

When no further application of the organic manure was made the effect diminished with time, so that by the 1948-49 season the treated plots showed little improvement in growth or susceptibility to hard-core over the controls except where soil sterilization had been carried out.

Analysis of cocoa husks and sheep manure reveals them as a rich source of some of the major plant foods as Table VIII will show. That they also supply trace elements may be taken for granted.

It will be seen that the total quantity of cocoa husks used on these plots supplied the equivalent of nearly 2 tons of sulphate of potash per acre and between 3 and 4 tons of sulphate of ammonia. By the end of the 1948-49 season the soil of plot 6, where cocoa husks had previously been applied, had only about 0·20 m.e. per cent. of exchangeable potassium and 0·04 per cent. of total nitrogen more than neighbouring control plots.

TABLE VIII.

Analysis of organic manures used on tomato plots. (*Block A.*)

	CaO		MgO		P_2O_5		K_2O		N	
	per cent.	lb. per acre.	per cent.	lb. per acre.	per cent.	lb. per acre.	per cent.	lb. per acre.	per cent.	lb. per acre.
Cocoa husks ..	0·36	242	0·87	585	0·94	632	2·89	1,942	2·57	1,727
Sheep manure ..	2·27	1,525	0·99	665	1·14	766	1·94	1,304	2·43	1,633
Raw sawdust ..	0·17	95	0·09	51	0·042	24	0·11	62	0·067	38
Rotted sawdust ..	0·78	524	0·82	551	0·082	55	0·17	114	0·47	316

Percentages are on the dry-matter basis.

In contrast to these rich organic manures, raw or rotted sawdust of low plant food content (Table VIII) did not prove beneficial. Both treatments, used with the standard fertilizer, produced symptoms of nitrogen deficiency although only slight in the case of the rotted sawdust.

4. *Effect of increased applications of potash and nitrogenous fertilizers.*

In view of the good results obtained with cocoa husks and sheep manure in quantities supplying heavy dressings of major plant foods, it was decided to increase the fertilizer quantities on certain plots. Three times the normal fertilizer was applied on two plots in Block A in the 1946-47 season. In one of the plots this treatment brought about a sufficient decrease in hard core to justify a continuation of the treatment (plot 11, Table VII). In the following season (1947-48) the standard fertilizer over the whole block was doubled to give a total of 2,000 lb. per acre of mixed fertilizer (see footnote, Table IV). The plots formerly treated with three times the standard fertilizer were now given the new standard amount of superphosphate and three times the standard of potash and nitrogen. The extra nitrogen was supplied as sulphate of ammonia and nitrate of soda. These plots were then receiving 1,000 lb. superphosphate, 1,200 lb. sulphate of potash, 1,010 lb. sulphate of ammonia, 300 lb. nitrate of soda and 300 lb. dried blood per acre. The effect of this fertilizer treatment repeated for two seasons is shown in Table VII (plots 11 and 14).

It will be seen that when the fertilizer was first increased in the 1946-47 season, plot 11 gave only 34 per cent. of commercial hard-core, compared with 87 per cent., 71 per cent. and 81 per cent. for the controls 7, 2 and 13. In 1947-48 when only one recording was made, in February at the height of the season, the figure for plot 11 was 64 per cent., whereas the controls were 96, 98 and 98 per cent. Of this 64 per cent., only 4 per cent. was badly affected fruit compared with 59, 51 and 63 per cent. for the controls. A February recording in the 1948-49 season showed the fruit almost free from the disorder on plot 11. Growth and yield for the season were comparable with the controls, but fruit was of much better quality and the plants had a more healthy appearance.

Plot 14 gave a slower response to heavy fertilizer applications. In the first season (1946-47) the amount of commercial hard-core on this plot was 62 per cent., about 20 per cent. lower than on the controls. In the next season, though the plot showed 99 per cent. of commercial hard-core, and by this rating was no better than the controls, only 33 per cent. was graded as bad hard-core, whereas the amount was 59, 51 and 63 per cent. in the three control plots. After sterilization with chloropicrin plot 14 still produced as much as 13 per cent. of commercial hard-core and 26 per cent. of slight hard-core, the benefit from the treatment being comparable with that obtained from the cocoa husk and sheep manure residues where chloropicrin was used (plots 6 and 5). Throughout the season, plants on plot 14 had a healthier appearance than had those on any other sterilized plot and less reject fruit.

A small experiment carried out on chloropicrin-treated soil in the 1948-49 season supplied additional evidence of the effectiveness of heavy potash and nitrogen applications in reducing the severity of hard-core. The treatments used, the percentage of commercial hard-core and the yield in pounds per plant are given in Table IX. Hard-core was recorded for two pickings, one in February and one in March.

TABLE IX.

Effects of different levels of applied potash and nitrogen on the amount of "Hard-core" on sterilized soil. (Block K.)

Plot.	Treatment.	Commercial "Hard-core" per cent.	Yield in lb. per plant.
K1	No fertilizer ..	47	7·2
K2	Standard plus 2 tons sulphate of potash per acre ..	25	9·6
K3	Standard ..	52	9·3
K4	Standard plus ½ ton sulphate of potash per acre ..	41	9·0
K5	Standard plus 2 tons sulphate of potash, plus a further application of the standard amount of nitrogen..	7	10·4

Results are expressed as number of fruits per cent.
The standard fertilizer treatment consisted of twice the quantities given in the footnote in Table IV.

The results in Table IX indicate little benefit from an extra ½ ton of sulphate of potash per acre, 41 per cent. of commercial hard-core being produced in plot K4 compared with 52 per cent. for the control K3. Two tons appeared to be more effective, giving 25 per cent. commercial hard-core (K2). Where extra nitrogen was applied with the high potash treatment (K5) the commercial hard-core was as low as 7 per cent. This treatment was associated with the highest yield.

The fact that heavy dressings of potash in addition to the standard fertilizer are more effective in reducing hard-core if extra nitrogen is applied at the same time, has also been demonstrated with plants grown in river sand.

Heavy dressings of nitrogenous fertilizers applied without extra potash have not brought about any decrease in the severity of the disorder.

Though increased applications of potash and nitrogen have been very beneficial, it must be pointed out that so far complete control of the disorder under the present system of tomato culture has not been achieved, even where the soil has been sterilized.

DISCUSSION.

Analyses recorded in this paper indicate that hard-core occurs in inverse ratio to the amount of available potassium in the soil and to the amount of potassium in the leaves and fruit of the tomato plants. The use of heavy applications of potash fertilizers has brought about a marked reduction in the amount of hard-core and where the heavy potash dressings have been supplemented by additional amounts of nitrogenous fertilizers, their effectiveness has been increased. Organic manures rich in potassium and nitrogen have proved very beneficial. Although these treatments have greatly improved the fruit quality they have not entirely eliminated the disorder.

Treatment of the soil with steam or chloropicrin and to a lesser extent with D-D or formalin has not only improved growth but also reduced the amount of hard-core. The results obtained with these sterilizing agents suggest that root diseases, by limiting the uptake of plant nutrients, may be a contributing factor in producing hard-core symptoms. Plants on sterilized soil have a more fibrous and better developed root system than have those on unsterilized soil.

The experimental work suggests that on the Nelson clay loam which has been used for tomato culture for many years, the principal factors causing hard-core are the low content of potassium in the soil and the prevalence of root diseases. The possibility that a soil of this texture may have a high fixing power for potash and also that the uptake of potassium may be affected by the high proportion of magnesium and calcium in the soil must be taken into account.

The failure to obtain in any of the experiments recorded in this paper a complete freedom from hard-core, may be due to some additional unidentified factor, for example, a trace-element deficiency. On the other hand, in certain commercial varieties of tomato, developed by plant breeders mainly on high yield characteristics, it is possible that the large crop which is produced in relation to the size of the plant, can mature normally only under very favourable soil conditions, seldom encountered in commercial practice.

SUMMARY.

1. A description is given of a widespread abnormal ripening of outdoor tomato fruit, known locally as "hard-core" to which heavy-cropping varieties are particularly subject.
2. Hard-core was found to be associated with a low potassium content of both leaves and fruit.
3. Certain areas in the Cawthron Institute tomato field were found to produce the disorder year after year while certain adjoining areas were relatively free. Susceptibility to hard-core was shown to be inversely related to the amount of exchangeable potassium in the soil.
4. The disorder was worse in plots receiving no fertilizer.
5. Soil sterilization with steam or chloropicrin was beneficial, but not to the extent of controlling the disorder on badly-affected areas.
6. Rich organic manures such as cocoa husks and sheep manure were very effective in decreasing the amount of hard-core, though a small amount was still present even after the application of 30 tons of dry matter per acre in addition to the standard fertilizer.
7. Hard-core was considerably reduced, but not eliminated by the use of heavy dressings of potash fertilizers.
8. Hard-core was further reduced, but not entirely eliminated by increased applications of nitrogenous fertilizers with the heavy potash dressings.

ACKNOWLEDGMENTS.

The author wishes to acknowledge the valuable assistance given by Dr. H. O. Askew and Mr. D. J. Stanton in the organization and conduct of the field experiments associated with the studies of hard-core. Thanks are also due to Miss J. Watson for the analysis of four soil samples and one sawdust sample reported in this paper and to Miss P. Walshe for the estimation of nitrogen in tomato fruit.

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(*Received* 7/1/50.)

THE STRAWBERRY APHID, *PENTATRICHOPUS FRAGARIAE* (THEOB.), IN NORTH WALES, WITH SPECIAL REFERENCE TO THE MAINTENANCE OF HEALTHY STRAWBERRY STOCKS IN NON-FRUIT-GROWING AREAS

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INTRODUCTION.

THIS paper reports the continuation of a study in North Wales of populations of the Strawberry Aphid, *Pentatrichopus fragariae* (Theob.), the only known natural vector of strawberry virus diseases. The original survey in two fruit-growing districts of Denbighshire (Thomas & Jacob, 1940) was extended to the western counties of Anglesey and Caernarvonshire, where fewer strawberries are grown, but where the slow rate of spread of viruses of the potato forms the basis of a local seed potato industry (Whitehead, 1943). It was considered that in so far as climatic factors reduced migration of the potato virus vector *Myzus persicae* Sulz. (Davies, 1934, 1935, 1936) they might similarly affect *P. fragariae*, and that the western counties might therefore be suitable also for the maintenance and propagation of healthy strawberry stocks.

POPULATIONS OF *P. fragariae* IN FRUIT GROWING DISTRICTS.

Thomas and Jacob (1940) recorded populations of *P. fragariae* in 1938 and 1939 at Holt, in the lower Dee Valley, and at Glan Conway, in the lower Conway Valley. The Holt district, which is geographically a part of the Cheshire plain, contains a large number of intensively worked holdings, many small in acreage, lying in and around the villages of Holt and Farndon. Serious disease and pest outbreaks are liable to affect the entire district. Glan Conway contains a smaller strawberry acreage, but the part of the valley where the crop is grown for fruit forms a compact locality where difficulties similar to those at Holt are experienced.

The survey of *P. fragariae* was continued at approximately monthly intervals from June, 1940, until November, 1945. The procedure consisted of traversing each plantation systematically and counting the aphids on one young leaf from each of 100 different plants (50 on very small plantations), the total being expressed as the number of aphids per 100 leaves (termed the "index-figure"). This method was the same as that adopted in 1938 and 1939 except that the writer used partly opened leaves instead of fully expanded young leaves. Fields examined in 1938 and 1939 and remaining in strawberries in 1940 were surveyed until they passed out of rotation, and new maiden plantations were introduced to the survey each year. Usually from three to six plantations were visited on each occasion.

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(*a*) *General population trends from 1938 to 1945.*

The writer is indebted to Dr. I. Thomas and to Mr. F. H. Jacob for permission to make further use of the data obtained in 1938 and 1939, which has enabled graphs to be constructed (Figs. 1 and 2) showing changes in populations of *P. fragariae* at Holt and Glan Conway during the period 1938-45. Each figure plotted is the geometric mean of all index-figures recorded on a given date (i.e. from plantations generally differing in variety and age) as determined by the logarithmic method suggested by Williams (1936). Geometric means are used because arithmetic means would be heavily biased by single exceptionally high counts at peak periods of infestation.

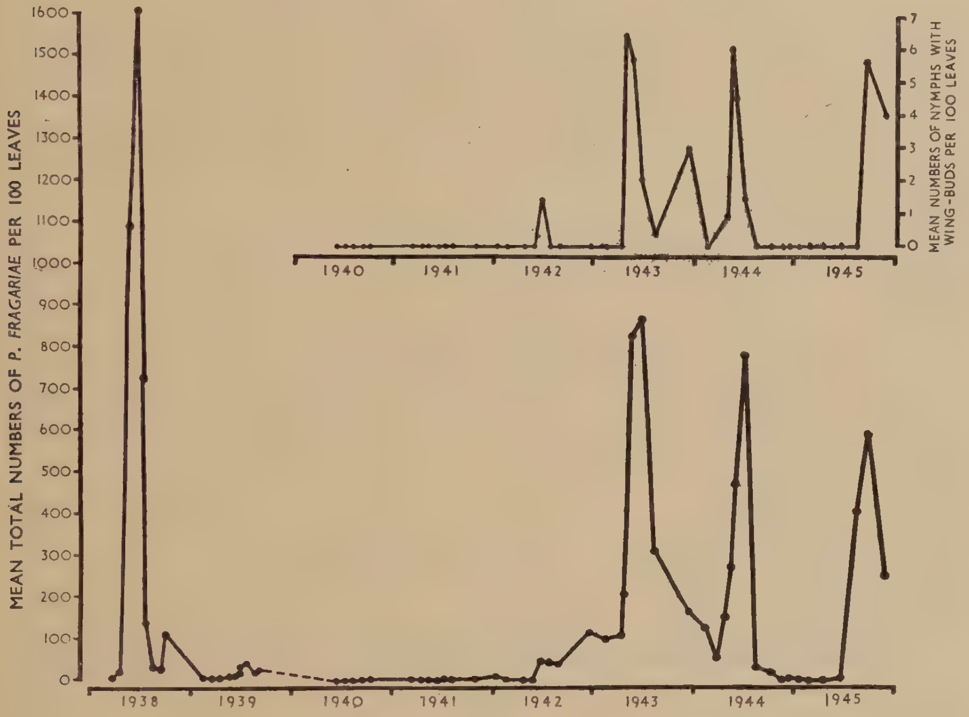

Fig. 1.

Populations of *Pentatrichopus fragariae* (Theob.) on strawberries at Holt, Denbighshire, in the years 1938-1945. (Figures plotted are geometric means of index-figures.)

The contrast between the high-infestation years of 1938, 1943 and 1944 and the low-infestation years of 1940-42 is great. Spring and summer populations in 1940 and 1941 were negligible, but rapid breeding in the autumn of 1942 and, especially at Glan Conway, during the following winter, resulted in the presence of large numbers of aphids early in 1943. These gave rise to enormous infestations in May and June of that year, followed shortly by a rapid decline in populations; but over-wintering was again considerable and the populations in May and June, 1944, were comparable with those of 1943. Another steep decline, beginning in late June, 1944, continued throughout the following winter, and the aphids were very scarce in the first-half of 1945. Not until late summer were appreciable numbers present.

(*b*) *Occurrence of alatae.*

There is sufficient evidence of the significance of winged generations of *P. fragariae* in the spread of strawberry viruses to justify their intensive study in particular districts. In the years 1936-38 Massee, Greenslade and Duarte (1938) and Greenslade (1941a) found alatae most numerous in Kent in May or early June, adult alatae being seen for one week only in 1937 and 1938. In this short period, however, migration was widespread and caused considerable spread of virus infection. Alatae also occurred during a more prolonged period in autumn, though in smaller numbers and without, apparently, achieving flight.

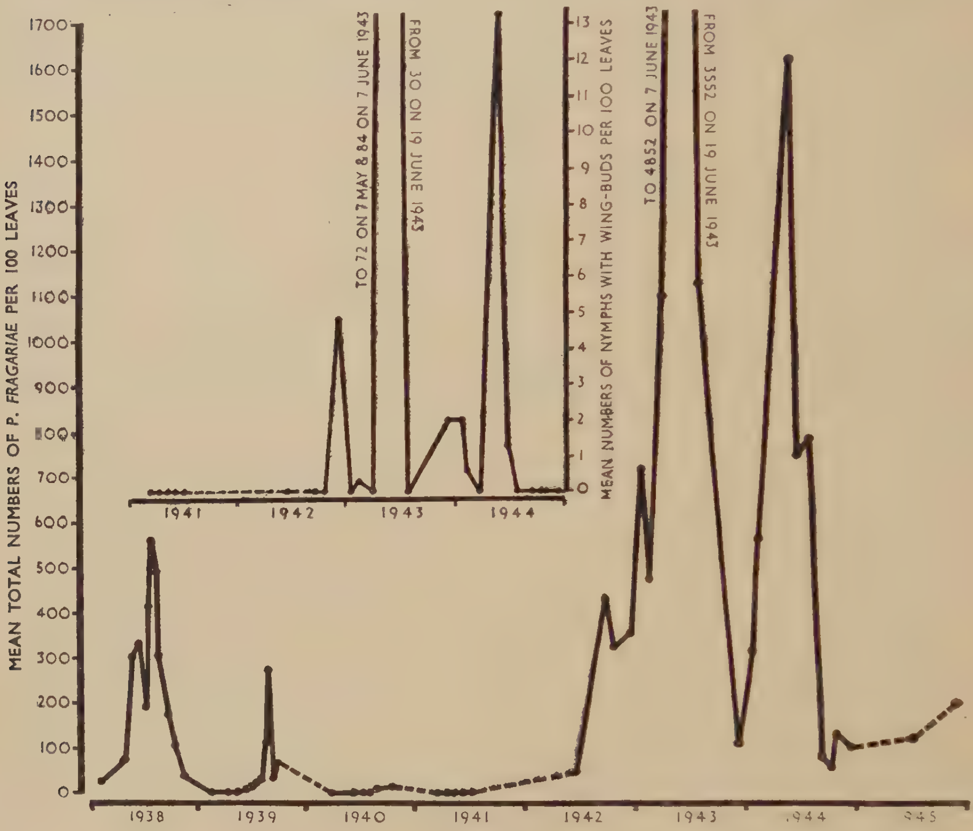

Fig. 2.

Populations of *Pentatrichopus fragariae* (Theob.) on strawberries at Glan Conway, Denbighshire, in the years 1938-1945. (Figures plotted are geometric means of index-figures.)

Thomas and Jacob (1940) found alatae plentiful at Holt and Glan Conway in 1938. None was found in 1939, although three nymphs with wing-buds were recorded at Holt in June of that year. The present records (see smaller graphs, Figs. 1 and 2) show that alatae were scarce or absent in 1940 and 1941, present in small numbers in the second half of 1942 and plentiful in 1943 and 1944. In 1945 they were not seen until October, when small numbers occurred at Holt. The main production of alatae in 1938, 1943 and 1944 occurred during late May and June, extending over periods of at least six weeks. It appears, therefore, that the spring and summer migration period is of longer duration in North Wales than in Kent. Each of these years was marked by great spread of virus infection in the Holt and Glan Conway districts (Thomas and Jacob, 1940; Whitehead and Wood, 1947).

Alatae of *P. fragariae* may also occur in North Wales in autumn and winter. Records of alatae in January and February at Glan Conway possibly reflect the mildness of the winter climate there as compared with the Dee Valley and the south-east of England.

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ERRATA.

Vol. XXVI, No. 1, pp. 25 and 34 :—
" Whitehead and Wood, 1947 " should read :
" Whitehead and Wood, 1946 ".

Summary 4, p. 58 :—
" matted rows up to 100 per cent." should read :
" matted beds up to 100 per cent."

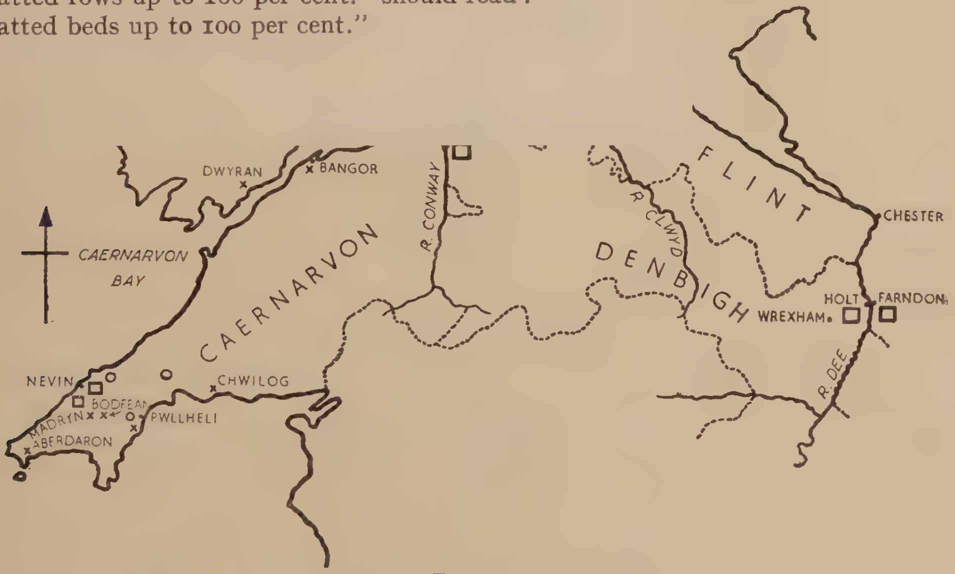
DWYRAN
BANGOR
R. CONWAY
CAERNARVON BAY
CAERNARVON
FLINT
CHESTER
R. CLWYD
DENBIGH
HOLT
FARNDON
WREXHAM
R. DEE
NEVIN
BODFEAN
MADRYN
CHWILOG
PWLLHELI
ABERDARON

Fig. 3.

Map of North Wales, showing location of survey centres.
☐ = commercial strawberry plantations or districts.
○ = private gardens.
× = experimental plots.

The plots were each about 200 square yards in area except for those at Aberdaron and Rhoscolyn, which covered 100 and 400 square yards respectively. The earlier plots were planted in 1938 with apparently virus-free stocks of Royal Sovereign from Eire, Northern Ireland and a local source, and were partly replanted with the same stocks during the following 4-5 years. The later plots were used mainly for the propagation of special nuclear strawberry stocks, and certain private holdings to which these were distributed were later included in the survey. All runners for planting were first either dipped in a nicotine wash or nicotine-fumigated in the glasshouse. The latter treatment is believed to have completely eliminated *P. fragariae.*

Each centre was examined for *P. fragariae* at intervals of 3 to 4 weeks in summer and less frequently in winter. At centres not known to be colonized the plants were searched individually, but after colonization the populations were recorded by the usual survey method.

TABLE I.

Populations of P. fragariae *on strawberries* (*Royal Sovereign*) *at Nevin, Caernarvonshire.*

Date.	Index figures.	
	(*a*) 29 rows planted spring, 1940.	(*b*) 20 rows planted spring, 1938.
1940		
27th June	No record	98
31st July	,,	411
30th August	,,	685
30th September	1,812	477
15th November	214	No record
10th December	76	,,
1941		
11th February	6	14
8th April	0	5
6th May	1	3
7th June	5	8
9th July	95	43
1942		
10th March	18	No record
10th April	0	,,
8th May	3	20
8th June	4	No record
14th July	212	,,
8th September	488	,,
21st October	180	,,
8th December	71	,,
1943		
20th January	16	No record
6th May	0	,,
29th July	144	No record
8th September	188	,,
15th December	12	,,

Index figures are numbers of *P. fragariae* per 100 leaves.

Two of the three original plots remained free from *P. fragariae* in 1938 and 1939, and the third, which became colonized in 1939 from an obvious nearby source, was replanted. Fig. 4 shows that the aphid was not found on any of the plots in 1940, 1941 or 1942. Other observations, however, showed that it was not absent from western Caernarvonshire during those years, the first record being obtained in a garden at Pwllheli on 21st June, 1940 ; counts were then begun on a 2-acre plantation at a mixed farm near Nevin (Table I), this being the only plantation of more than garden size in that part of the county. The maximum index-figures for 1940 and 1941 exceed any corresponding figures from Holt or Glan Conway. No winged forms of *P. fragariae* were seen at Nevin in 1940 or 1941, but a single nymph with wing-buds was found on 14th July, 1942.

In 1943, when alatae were so plentiful in other areas, there was a corresponding increase in the west. On 21st May eleven nymphs with wing-buds were found on garden strawberries at Morfa Nevin, Caernarvonshire, and an alate adult in a garden at Beaumaris, Anglesey. A nymph with wing-buds was found at the larger centre near Nevin on 29th July. Those experimental plots which became colonized probably did so as the result of migration under the favourable conditions of 1943.

The first observation of *P. fragariae* on an experimental plot was made at Benllech on 29th June, 1943, and later that year another plot and two private runner-beds were found colonized. Not until 1944, however, were populations large ; summer index-figures that year included 462 and 358 at two Caernarvonshire

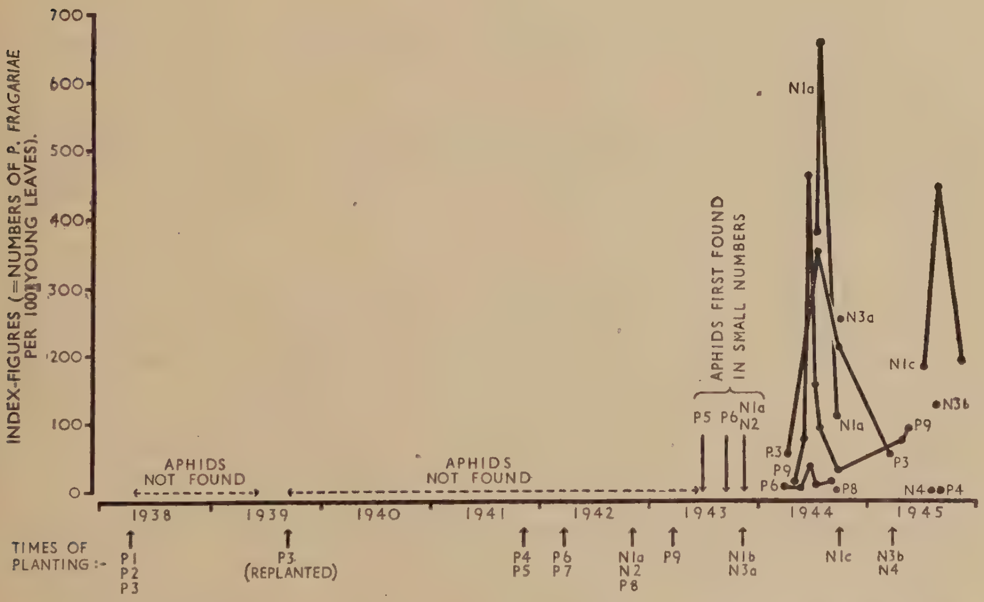

Fig. 4.

Occurrence of *Pentatrichopus fragariae* (Theob.) on isolated experimental plots and runner-nurseries in North Wales in the years 1938-1945.

P1–3 = plots established 1938-39.
P4–6 = later plots in Anglesey.
P7–9 = later plots in Caernarvonshire.
N1–2 = private nurseries in Caernarvonshire.
N3 = private nursery in Denbighshire.
N4 = private nursery in Anglesey.
(Small letters denote annual plantings.)

plots, 54 at the Dwyran plot, and 664 and 255 at private runner-beds in Caernarvonshire and Denbighshire. The 1945 figures were again appreciable. Adult alatae were not seen in the west in 1944 or 1945, but in 1944 a few nymphs with wing-buds were found at two Caernarvonshire centres on 3rd May and 19th June. These observations showed that the aphid, once introduced into westerly districts of North Wales, could persist and attain populations comparable with those often present in established strawberry-growing areas. The plots at Aberdaron, Rhoscolyn, Bodfean and Valley, however, remained free from *P. fragariae* throughout the period of their use (i.e. until the end of 1945) ; nor was the aphid found on private runner-beds planted at Morfa Nevin in autumn, 1943, and at Bryngwran (Anglesey) in spring, 1945.

SOME ENVIRONMENTAL FACTORS INFLUENCING POPULATIONS OF *P. fragariae.*

(*a*) *Winter weather conditions.*

The present survey confirmed that severe winter weather can greatly reduce populations of *P. fragariae* (Greenslade, 1941b), thereby preventing the development of large infestations in the following spring. Weather records from Eaton Gardens, near Chester, some four miles from Holt, show that the winters 1937-38, 1942-43 and 1943-44 were generally mild. Each was followed by a season of high aphid populations (Fig. 1). No winter survey work was done in 1937-38, but in the other two winters the aphids bred actively from December to March and were numerous at the beginning of spring. In contrast, the winters 1939-40, 1940-41 and 1941-42 were severe, and were followed by seasons of low populations. Small numbers survived, however, for the populations again rose in 1942 to reach a fairly high level by late autumn.

In the more normal winters 1938-39 and 1944-45, January frosts probably caused a reduction in over-wintering aphids leading to smaller spring and early summer populations ; but the effect of any increase in predators following the previous seasons' high aphid infestations must also be considered.

(*b*) *Conditions affecting wing-formation and flight.*

Thomas and Vevai (1940), investigating a series of trapping records, concluded tentatively that the most important factor favouring the production of winged forms in Aphididae in general is the amount of sunshine during May. A comparatively sunny May, with large daily fluctuations in temperature and low relative humidities, appeared to accelerate wing-formation. To apply these conclusions to the present survey an analysis was made of weather records from April to June each year from two stations near Holt, and in Table II are shown periods likely to have favoured wing-formation. Outstandingly favourable periods are indicated by an asterisk.

That the periods shown for 1938, 1943 and 1944 did provide suitable conditions for wing-formation in *P. fragariae* is suggested by their apparent relationship to the times of appearance of nymphs with wing-buds in those years ; but it would then appear that all the other years of the survey, with the doubtful exception of 1940, offered conditions for wing-formation at least as favourable as those of the three migration years.

The same workers also examined weather conditions on days of peak aphid migrations, and suggested arbitrary standards of temperature, relative humidity

Table II.

*Periods favourable for development of alate aphids in the Holt district of Denbighshire. Periods outstandingly favourable are indicated by *.*

Year.	Periods.	Nymphs with wing-buds.	Alatae.
1938	4th April-11th May*	Present late April	Present 26th May-5th July
1939	7th-21st April 19th May-10th June*	Two on 6th June One on 16th June	None
1940	25th April-14th May 2nd-21st June	None	None
1941	22nd April-15th May	None	None
1942	12th April-10th May*	Three on 22nd June	None
1943	9th-21st April 2nd-29th May	Numerous 27th April Numerous 17th May Occasional 3rd August	Numerous 17th May Fewer 21st June Occasional 3rd August
1944	16th April-30th May*	Present 28th April Numerous 11th and 26th May Present 26th June Absent 11th August	Numerous 26th May Present 26th June Absent 11th August
1945	6th-27th April	None in spring or summer	None in spring or summer

and wind velocity suitable for flight. These standards were based on meteorological station records, which often bear no close relationship to microclimatic conditions within growing crops (Broadbent, 1949a). Recent work, moreover, diminishes the importance of high relative humidity as a factor limiting aphid flight (Broadbent, 1949b). Nevertheless, these standards do indicate days and periods which are mainly warm, sunny and calm, when more aphid flight may be expected than under cold, dull and windy conditions. They were therefore used in an analysis of weather conditions at Holt in the months of May, June and July throughout the present survey, and this showed that each of the non-migration years probably provided climatic conditions favouring appreciable flight of *P. fragariae*. Of the migration years, 1938 appears to have been poorest for flight. Two of the non-migration years, 1941 and 1945, were somewhat similar to 1938, whereas the other three, particularly 1940, were distinctly more favourable for flight.

Thus, in three of the eight seasons discussed, a migration of *P. fragariae* causing serious spread of virus diseases actually occurred in the Holt district. Two other years, 1939 and 1942, apparently offered good conditions both for development and flight of alatae, and might have been years of high virus spread had aphid populations been larger. Two of the remaining years, 1941 and 1945, were favourable for the production of alatae and certainly offered some periods favourable for flight. 1940, though of questionable suitability for wing-formation, provided excellent conditions for flight, so that a small number of alatae developing that year might have migrated widely. It is concluded that in a concentrated strawberry-growing area such as the Holt district there is danger of large-scale spring or early summer migration of *P. fragariae* in almost any year, provided sufficient numbers of aphids are present.

(*c*) *Predators and parasites.*

In each of the high-infestation years there was a very rapid decline in populations of *P. fragariae* beginning, in many plantations, as early as the second half of June

(Figs. 1 and 2). Thomas and Jacob (1940) stated that the decrease in populations in 1938 was chiefly due to the action of predators and parasites.

Dicker (1944) described two species of beetles commonly occurring as predators of the strawberry aphid in southern and midland counties of England, and Massee (1946) reported a species of *Aphidius* parasitizing *P. fragariae.*

In the years 1940-45 the writer did not record predators of the strawberry aphid, but observed the action of a parasitic fungus, *Empusa Planchoniana* (Cornu) Petch,* which was probably identical with a species seen by Thomas and Jacob (1940) in 1938. *P. fragariae* attacked by this fungus assumed an orange or red coloration. The fungus was first noted at Holt and Glan Conway in early June, 1943, when the aphids were plentiful and much migration had already occurred. It played a major part in the reduction of populations in the next four or five weeks, but after the end of July aphids were rarely found newly parasitized, and moderate populations persisted in both districts throughout the autumn. In 1944 the fungus was first seen at Glan Conway in late May, when migration of alatae was in progress, and at Holt in late June, about a month after the main production of alatae. Its activity had largely ceased by the end of August, but at Glan Conway the aphid populations did not fall below about 50 per 100 leaves (7th September) and increased in late autumn.

Thus *E. Planchoniana*, though an important factor in reducing high summer populations of *P. fragariae*, did not become active early enough to interfere with the spring migration of alatae. It is of interest that the fungus was active throughout both districts in 1943 despite the great scarcity of *P. fragariae* in the three preceding seasons.

(*d*) *Variety ; age of plantation.*

Thomas and Jacob (1940) examined a two-year-old strawberry plantation containing alternating plots of Huxley Giant and Tardive de Leopold, and found that the peak index-figure for *P. fragariae* on the Huxley Giant was 9,784, whereas on the Tardive de Leopold it was 678.

In later years of the survey Huxley Giant was so generally grown that comparisons with other varieties could not easily be made, but a few are given in Table III. The plantations in each pair were of equal age, those in pairs (*a*) and (*b*) being adjacent ; those in pair (*c*) were in different fields about 150 yards apart.

In each comparison Huxley Giant came through the winter with the higher aphid population, although in two it had earlier carried the lower. In each case the population on Huxley Giant continued to rise in the spring and greatly exceeded that on the other variety. Some of the largest counts of alatae obtained were from plantations of Huxley Giant.

During recent years this variety had become popular in the Holt district, as elsewhere, because it offered reasonable promise of a crop when reliable stocks of superior quality varieties were difficult to obtain. Quite apart from its significance as a partially virus tolerant or " carrier " variety, however, its hardy foliage clearly provides a favourable winter habitat for the strawberry aphid. This results in high spring populations, constituting a major source of new infestations by alatae. New varieties of similar growth-habit to Huxley Giant might well be open to the same objection.

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* Kindly identified by the late Mr. T. Petch.

Table III.

Comparative populations of P. fragariae *on different varieties of strawberry.*

	1942.		1943.				
	27/8	17/12	12/2	5/4	17/5	21/5	3/8
(*a*) Oberschlesien	75	33	0	6	128	566	302
Huxley Giant .. .. (Planted autumn 1940)	46	112	88	145	628	1,130	176
	1942.	1943.					
	9/12	18/1	15/2	1/4	7/5		
(*b*) The Duke	507	371	165	368	?		
Huxley Giant .. .. (Planted autumn 1940)	350	728	636	1,174	1,986		
	1943.	1944.					
	1/12	11/2	27/3	28/4	26/5	26/6	11/8
(*c*) Royal Sovereign	122	208	41	62	590	898	?
Huxley Giant .. .. (Planted autumn 1942)	174	288	212	397	1,445	3,584	77

The numbers are Index-figures.

Table IV compares plantations of different ages. Comparison (*a*) is between two long, narrow, adjacent plantations, whereas those in (*b*) were separated by a 30-yard belt of vegetables. The records in (*a*) commenced more than a year after the planting of the younger plot, and any difference in incidence of predators and parasites between the two plots might be expected to have disappeared; yet there was a much higher aphid population on the younger plot, the difference persisting through the high-infestation period of 1943. The records in (*b*) show a similar trend in the same year, the population on the younger plot overtaking and greatly surpassing that on the older. In each example the younger plot showed the more vigorous

Table IV.

Comparative populations of P. fragariae *on plantations of Huxley Giant of different ages.*

	1942.	1943.				
	17/12	12/2	5/4	27/4	17/5	21/6
(*a*) Planted autumn 1939	480	402	?	788	?	?
Planted autumn 1941	876	1,208	2,452	4,036	9,140	254
		1943.				
		5/4	17/5	21/6	3/8	1/12
(*b*) Planted autumn 1940		154	708	?	572	114
Planted autumn 1942		48	1,452	3,390	742	174

The numbers are Index-figures.

growth, with more young foliage, and the leaves on which counts were taken were more shaded by other leaves than on the older plot. These observations suggest that young, actively growing plants provide a more congenial environment for the strawberry aphid than do older plants of poorer vigour, and point afresh to the need for routine insecticidal treatment of runners before planting and also of established plantations, however healthy the plants may appear.

DISCUSSION.

The work described shows the extent to which *Pentatrichopus fragariae* has existed in recent years as an economic pest in the two main fruit-growing districts of North Wales. With present-day strawberry varieties in districts of intensive cultivation, infestations of the size recorded produce serious spread of virus diseases, with especially drastic losses resulting from the infection of uncropped maiden plantations. Natural factors provide no more than a temporary check. The parasitic fungus *Empusa Planchoniana* reduces heavy infestations but does not interrupt migration, and a single congenial season can enable the aphid to recover from the effects of severe winter weather.

A close similarity to some of the present results is shown by more recent observations in North Wales by Mr. F. H. Jacob.* The hard winter weather of January and February, 1947, caused populations to be negligible in the spring and summer of that year, leading to a corresponding scarcity of predators and parasites. This, together with a mild winter in 1947-48, permitted a steady increase in aphid populations from the autumn of 1947 to the following spring, when warm, dry weather favoured the further development of enormous infestations. Widespread migration occurred and was largely completed by mid-May, resulting in very severe virus infection of maiden plantations. Over-wintering was again appreciable in 1948-49 and led to renewed heavy infestations in 1949. This sequence of events, resembling that of the period 1942-44, suggests that the time taken for predators and parasites to recover from a period of low activity may be a factor tending to produce abnormally high aphid populations for at least two successive seasons, provided that hard winters do not intervene.

An essential requirement in the two fruit growing districts surveyed is a routine annual control treatment to prevent the main migration of alatae. Such a treatment should be technically simple and efficient and should be applied to all plantations, large and small, including garden plots. Trials at Holt with a nicotine fumigating machine during the past five seasons sometimes gave good results, but the need for suitable temperature and other weather conditions often prevented timely application. Timing is important, owing to the appreciable year to year variation in the migration period, and makes local specialist supervision desirable. Newer insecticides now available may prove superior to nicotine, especially if combined with an efficient spraying technique less costly than fumigation. Another routine treatment which should be adopted is the dipping of runners in an insecticide before planting (Greenslade and Massee, 1939), to ensure as far as possible that maiden plantations start free from aphids.

The strawberry aphid can readily be introduced into a new district by planting undipped runners, and can afterwards maintain itself as in other areas. Indeed, the figures in Table I suggest that in hard winters more *P. fragariae* may survive in districts such as western Caernarvonshire than farther inland. Certain plots used in the present survey did remain uncolonized in 1943 and 1944, but any considerable extension of strawberry-growing in western parts of North Wales would almost certainly increase the number of aphid foci and so reduce the effective isolation of many plantations. Careful attention to routine control measures, together with the introduction of none but the healthiest strawberry stocks, is therefore essential if the serious problems besetting older strawberry-growing districts are to be avoided. From the cultural standpoint certain western districts, particularly in Caernarvonshire and south Anglesey, offer favourable conditions for either fruit or runner production. Climatic conditions favour late summer or early autumn planting which, in turn, should permit specialization for early markets.

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* Received by private communication.

SUMMARY.

1. Earlier workers found that spring populations of *Pentatrichopus fragariae* (Theob.) in two intensive strawberry-growing districts of North Wales were high in 1938 and lower in 1939. The present paper shows that the aphid was extremely scarce in 1940 and 1941, following severe winters. Breeding in the autumn of 1942 and during the following mild winter resulted in high populations in 1943. Populations were again high in 1944 but low in 1945.

2. Plantations of the strawberry variety Huxley Giant carried larger populations of *P. fragariae* than did comparable plantations of certain other varieties. Larger populations occurred on young than on older plantations of Huxley Giant.

3. The fungus *Empusa Planchoniana* (Cornu) Petch is a frequent parasite of *P. fragariae*, but in three seasons of high aphid infestations it did not prevent widespread migration by alatae.

4. Migration by alate *P. fragariae*, causing serious spread of virus infection, occurred in 1938, 1943 and 1944. Alatae were plentiful for at least six weeks, mainly in late May and June. From an examination of meteorological data it is concluded that such migration is likely to occur in the lower Dee Valley in almost any year in which populations of the aphid are large.

5. Experimental plots in non-strawberry-growing districts of Caernarvonshire and Anglesey remained free from *P. fragariae* in 1940, 1941 and 1942, but five of nine plots were colonized following migration in 1943. It is concluded that an extension of strawberry-growing in these districts would necessitate the same measures of aphid control that are required elsewhere.

ACKNOWLEDGMENTS.

The writer wishes to express his thanks to Dr. T. Whitehead and Dr. I. Thomas, under whose direction this work was done; to Mr. F. H. Jacob, for permission to use recent unpublished observations; to Mr. N. F. Barnes, of Eaton Gardens, Chester, and to R.A.F. Station, Sealand, for meteorological data; and to many growers in North Wales for their interest and generous co-operation. This work was started when the writer held a Studentship of the University of Wales and was continued with the aid of a grant from the Agricultural Research Council.

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RUBUS STUNT: A VIRUS DISEASE

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A DWARFING disease of Phenomenal berry (*Rubus loganobaccus* Bailey), Himalaya berry (*R. procerus* Muell) and Parsley-leaved blackberry (*R. laciniatus* Willd.) has been common in south-east England for a number of years and, more recently, similar symptoms have been observed on Loganberry (*R. loganobaccus*), wild blackberry (*R. fruticosus* L.), raspberry (*R. idaeus* L.), English red-black hybrid raspberry (*R. idaeus* × *R. occidentalis* L.) and other hybrid *Rubus* berries including Thornless Loganberry, Boysenberry, Thornless Boysenberry, Youngberry, Laxtonberry, and Merton Early. The disease has been known to growers by several different names —Reversion, Loganberry Dwarf, etc., but " Reversion " is considered unsuitable as it implies a change to an earlier or more primitive type and " Loganberry Dwarf " assumes that the disease is identical with a somewhat similar disease reported from America (Zeller, 1927). As this identity is by no means certain, the distinctive name Rubus Stunt is proposed for the disease and will be used in this paper.

The first record in the literature of the occurrence of the disease in Great Britain is on Himalaya berry and Parsley-leaved blackberry (Wormald and Harris, 1932). Unpublished work by R. V. Harris and M. E. King showed that the disease could be transmitted by grafting from Phenomenal berry to Phenomenal berry, Loganberry, Parsley-leaved and Bedford Giant blackberry; and while, when the disease was first noted, it was common on Phenomenal berry as well as blackberry, the true Loganberry was rarely infected (R. V. Harris, private communication). Recently, however, the disease has become increasingly prevalent in Loganberries and has seriously interfered with the commercial cultivation of Phenomenal berry, Loganberry and blackberries in southern England. At East Malling Research Station outbreaks of Stunt have occurred on Loganberry plantations since 1944 and, despite roguing, have extended to such a degree as to necessitate the abandonment of certain experimental plots. Serious outbreaks were reported on two commercial plantations of Loganberry in Kent in 1945 and since then outbreaks have been reported from other counties in southern England.

In 1944 two raspberry plants, of the variety Lloyd George, that showed symptoms similar to those produced by Stunt on loganberry were found in a garden near Maidstone, Kent. These plants died before it was possible to confirm the cause of the disease by grafting to loganberry. Specimens of red-black hybrid raspberries were found at East Malling in the same year with similar symptoms, but efforts to graft these to loganberry were unsuccessful. Since then, the disease has been found at East Malling in fruiting and/or propagation beds of Malling Enterprise, Malling Landmark, Malling Notable, Malling Promise, Lloyd George, and eleven unnamed seedling raspberries. It has also been seen in commercial plantations of Norfolk Giant and Malling Landmark raspberry in Kent, and in private gardens elsewhere.

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THE DISEASE.

The symptoms of Rubus Stunt on loganberry are easily recognizable. The new canes are weak, short and thin and much more numerous than normal (Fig. 1). Axillary buds near the base of the cane sometimes develop into branches and thus accentuate the bushy appearance of the plant. In the following season this weak cane generally fails to flower at all, or produces so few flowers that there is practically no crop of fruit. In recently infected plants the fruiting canes tend to have shortened internodes and often more than one bud develops at each node (Fig. 2). The flowers on the fruiting laterals are less numerous than normal and may be malformed—the floral parts, especially the sepals and carpels, becoming foliar. The effects of disease are progressive ; in successive years the new cane is more stunted until only a dense bush of numerous very thin, short canes remains.

Affected plants are thus easily recognized when the disease is in an advanced stage, especially if the fruiting cane is removed (Figs. 7 and 8). In order to control the spread of the disease, however, it is necessary to identify infected plants at the earliest possible moment, and this can be most readily done in early summer (usually in May), when the new canes on normal plants are about six to twelve inches long. At this stage the leaves on the new canes of infected plants are noticeably paler in colour than normal and can be readily identified. Closer examination will then show that, instead of the normal three to eight new canes (depending to some extent on the age of the plant), infected plants have ten, twenty or more thin new canes. The leaves on newly infected plants may be red-purple or bronze when the canes first appear but rapidly change to a pale yellowish green. At this period the leaves on healthy plants are still dark red-purple and although they gradually turn green, the colour remains of a darker shade than that on infected plants.

Symptoms on Phenomenal berry are similar. On Himalaya berry and Parsley-leaved blackberry (Fig. 3) the deterioration in vigour is rather slower and an infected Himalaya plant, especially if it is a large one, several years old when infected, may continue to produce a number of moderately vigorous canes (as well as many weak ones), for two or three years after infection. Although deterioration is slower than in loganberry and Phenomenal berry it is nevertheless speedy and progressive and infected plants soon become quite unproductive. Blackberry leaves are not pigmented to the same extent as loganberry leaves and there is usually no obvious difference in colour of foliage to aid in identification of diseased plants. Abnormal flowers, with the floral parts modified into leaves, sometimes occur in Parsley-leaved blackberry.

Symptoms in Thornless Loganberry, Boysenberry, Thornless Boysenberry, Youngberry and Laxtonberry and on Merton Early blackberry are similar to those on Parsley-leaved blackberry. On wild blackberry, the fruiting laterals are short and often several are produced at each node ; the flowers are few and the new canes are numerous, short and thin.

In raspberry, the symptoms depend to some extent on the variety infected. Generally the buds on the fruiting canes are slower in starting into growth in the spring, and the laterals which are produced remain shorter and produce fewer flowers. Thus infected plants can often be detected by inspection when the fruiting laterals on healthy plants are about six inches long. Some varieties of raspberry show a marked tendency to produce a proportion of abnormal flowers. Sometimes the sepals are slightly longer than normal or the tips more leafy and in extreme instances the sepals are modified into large leafy structures (Fig. 4). In other flowers the sepals are normal but the axis continues growth so that the other floral parts become raised on a short stalk ; the carpels are then generally foliar and sterile. Extreme abnormality of flowers is rare but is commoner in some varieties (e.g. Malling Landmark) than in others (e.g. Norfolk Giant). Occasionally the tips of sepals on *healthy* plants, especially on the lower laterals on a cane, may be somewhat leafy, so that slightly abnormal sepals are of doubtful diagnostic value.

In some varieties (e.g. Malling Landmark) the new canes on infected plants are very numerous and weak, while in other varieties (Baumforth's Seedling B, Lloyd George, Malling Enterprise, Malling Notable, Malling Promise), this character is less pronounced but still evident (Fig. 5). In Norfolk Giant the number of new canes produced is sometimes not much above normal and the new canes may grow to two feet or more in height. In such cases, diagnosis of infection is difficult and, if inspected during the summer, the plants merely appear weak ; diagnosis is easier early in the season when the late development of fruiting laterals can be seen and the shortness of laterals is not obscured by the growth of neighbouring healthy plants.

In raspberry propagation beds (" cane nurseries ") where no fruiting cane is allowed to develop (Wood, 1949) advanced stages of Stunt are easily recognized. More root buds than usual develop into shoots, and tufts of thin, weak shoots are produced (Fig. 6). Tufts of weak shoots are sometimes formed when canes are damaged during hoeing, etc., but if the tuft is dug up it will be found to consist of a single shoot which branches below ground level, and may thus usually be distinguished from Stunt.

TRANSMISSION EXPERIMENTS.

A number of one-year-old loganberry plants were planted out-of-doors in March, 1945, and five of them were grafted in June with scions from a loganberry affected with Stunt. The grafts were " bottle grafts ", the scions being inarched to canes of the indicator plants about three inches from the tip and the cut ends of the scions being placed in tubes of water which were supported in a suitable position on bamboo canes.* Five control plants were similarly grafted with similar scions from a normal loganberry. Several of the grafts appeared to " take " but afterwards died and all the grafts were therefore repeated in August, 1945. No symptoms appeared on any of the plants during 1945, but in May, 1946 all those grafted with Stunted scions showed symptoms of Stunt whereas the controls remained normal. The number of new canes on the infected plants ranged from six to nineteen with an average of 12·8, the controls having from three to five new canes with an average of 4·6. Five ungrafted plants had four to six new canes with an average of 4·8 canes. In October, 1946, the infected plants were obviously affected by Stunt and in May, 1947, symptoms were advanced (Fig. 7).

Four loganberry plants were grafted in 1945 with scions from a naturally infected wild blackberry. All four developed Stunt in 1946.

Four Parsley-leaved blackberry plants were grafted with scions from infected loganberry in 1945 and although some of the grafts appeared to have taken, no symptoms appeared on the blackberries in 1946. The plants were regrafted with infected scions in 1946 and three of the four had developed symptoms of Stunt by June, 1947. Five control plants grafted with scions from normal loganberry remained

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* The method is one developed for use on raspberries by C. H. Cadman (unpublished work).

TABLE I.

Summary of grafting experiments.

Indicator.	Infector.	Date of graft.	Date of symptoms.	Plants grafted.	Plants showing symptoms.
LOGANBERRY	Stunted Loganberry	1945	1946	5	5
BLACKBERRY	,,	1945 and '46	1947	4	3
RASPBERRY					
Norfolk Giant	,,	1945 and '46	Doubtful '47 Definite '48	5	4
Norfolk Giant	,,	1946	Definite '49	2	1
Baumforth's Seedling B	,,	1946	Slight '47	2	1
Lloyd George	,,	1946	Slight '47 Definite '48	2	1
Malling Enterprise ..	,,	1947	1949	5	4
Malling Landmark ..	,,	1947	1948	5	5
Malling Promise ..	,,	1947	1949	5	3*
LOGANBERRY	Stunted Wild Blackberry	1945	1946	4	4
	Stunted Raspberry A.	1947	1948	2	2
	Stunted Raspberry B.	1947	1949	2	1
	Stunted Raspberry C.	1947	1949	2	1
Control Grafts.					
LOGANBERRY	Normal Loganberry	1945	—	5	0
	Normal Wild Blackberry	1945	—	3	0
RASPBERRY					
Norfolk Giant	Normal Loganberry	1945	—	6	0
Malling Enterprise ..	,,	1947	1947	4	1
Malling Landmark ..	,,	1947	—	4	0
Malling Promise ..	,,	1947	—	4	0

* Two showed only slight symptoms.

normal. It is thought that infection was caused by the grafts made in 1945, and that diagnostic symptoms took two years to develop on Parsley-leaved blackberry.

Five Norfolk Giant raspberry plants were grafted with scions from infected loganberry in 1945 and six control Norfolk Giant plants were grafted with normal loganberry scions. No symptoms appeared on the raspberry plants in 1946 and they were regrafted in 1946. In 1947 the tips of the sepals of four of the five plants grafted with Stunt infectors were considered to be slightly foliar; the remaining plant (i.e. plant 4), and the control plants remained normal. Scions were taken from three of the Norfolk Giant plants and grafted to loganberry in 1947. These plants were (*a*) the grafted plant which appeared normal (plant 4), (*b*) a grafted plant on which calyx segments had leafy tips (plant 5) and (*c*) one of the control plants (plant 6).

The loganberries grafted with scions from plants 4 and 6 remained normal while the one grafted with a scion from plant 5 developed Stunt in 1948. It was thus confirmed that the Norfolk Giant plant with leafy sepal tips was infected with Stunt.

By autumn 1947 the number of new canes on the grafted Norfolk Giant plants was greater than that on the control plants. In 1948 these plants had from six to twenty-three fruiting canes (including eight on plant 4 which still appeared normal) with an average of 14·4 (16·0 omitting plant 4). The control plants had from two to twelve fruiting canes with an average for the six plants of 6·3 canes per plant. The grafted plants (except plant 4) were late in beginning growth in the spring (Figs. 9 and 10) and the fruiting laterals remained relatively short. It appears from this experiment that Norfolk Giant raspberry can be infected with Stunt and that the incubation period of the disease, when transmitted by grafting, is either two or three years (depending on whether infection resulted from the 1945 or 1946 grafts). The next experiment supports the view that infection was caused by the grafts made in 1945 and that symptoms on Norfolk Giant take three years to develop.

In 1946, two plants of Norfolk Giant, two of Lloyd George and two of Baumforth's Seedling B (all in pots) were grafted out-of-doors by the cane-inarching method (Harris, 1940) to loganberry plants with Stunt. In the following year one of the Lloyd George and one of the Baumforth B plants developed slight symptoms of Stunt and one of the Baumforth B plants was dead. The raspberries were back-grafted to healthy loganberries to determine whether they were infected and the results showed that one plant of Norfolk Giant and both plants of Lloyd George had Stunt. The remaining Norfolk Giant plant and the Baumforth B plant produced no effect on the healthy loganberry. To prevent the raspberry plants becoming pot-bound, they were planted out-of-doors in autumn, 1947. In 1948 the Norfolk Giant plant known to have Stunt showed no symptoms, but it developed definite symptoms in 1949 which accords with the previous indication that symptoms in this variety take three years to develop. The other Norfolk Giant plant appeared normal. One of the Lloyd George plants showed symptoms in 1948 and 1949, but the other, although not vigorous, could not be definitely diagnosed as having Stunt. The Baumforth B plant was very weak and lacked vigour, but could not be diagnosed as having Stunt. It may, however, have been infected as indicated by the slight symptoms which developed in 1947. The failure to confirm infection by grafting to loganberry may be ascribed to graft failure.

In 1947 five plants of Malling Enterprise, five of Malling Landmark and five of Malling Promise were inarched to loganberries with Stunt. In 1948 four of the Landmark plants showed definite symptoms of Stunt but all the flowers appeared to be normal. The remaining plant of Landmark and all the Enterprise and Promise plants appeared normal, though there were a few slightly abnormal flowers on one of the Enterprise and two of the Promise plants. In 1949 all five Landmark plants had Stunt and two of them had very abnormal flowers; four Enterprise plants had Stunt and one was dead; one of the Promise plants had definite Stunt, two showed slight and somewhat doubtful symptoms and one was apparently normal.

In 1947 two plants of each of three unnamed raspberries apparently naturally infected with Stunt were grafted to normal loganberries. In 1948 the loganberries grafted to one of the seedlings had Stunt; those grafted to the second seedling showed only slight symptoms but in the following year one had definite Stunt ; those grafted to the third seedling were apparently normal in 1948 but one had Stunt in 1949.

It is concluded from these experiments that Stunt is a graft-transmissible disease and, as no visible pathogen has been detected, the causal agency would appear to be a virus. As blackberries and raspberries have been infected from loganberry, and loganberry has been infected from naturally infected wild blackberry and raspberry, it appears that Stunt in each of these hosts is caused by the same virus. It is assumed that similar symptoms seen on other *Rubus* varieties are produced by the same virus. It is also concluded that definite symptoms of Stunt normally develop on loganberry in the season following the one in which the plants become infected, and that very severe symptoms develop in the season after that. Occasionally, however, symptoms may fail to develop in the year following infection, or may be so slight as to make diagnosis difficult. In such exceptional cases, diagnosable symptoms do not develop until the second year following infection. With blackberries clear symptoms do not normally develop until two years after infection. With raspberry the incubation period varies with the variety. Thus with Malling Landmark raspberry, symptoms are easily recognized and normally develop in the year following infection ; symptoms on Lloyd George, Malling Enterprise and probably Baumforth's Seedling B take two years to develop, while Malling Promise and Norfolk Giant develop, at most, only very slight symptoms in two years and diagnosable symptoms in three. The symptoms on Norfolk Giant too are less extreme than those on other varieties.

CONTROL.

The vector of the Stunt virus has not yet been discovered. Some viruses of *Rubus*, however, are known to have aphid vectors and winter tar oil spraying of *Rubus* is advised as a precautionary measure to keep down the aphid population. In loganberry plantations this, combined with careful roguing of infected plants as soon as they are detected, has generally been sufficient to control the disease at East Malling. On two occasions when serious infection has occurred in loganberry at East Malling, infected wild blackberries were found in adjacent hedges and infection may have spread from them. Careful examination of wild *Rubus* in the proximity of loganberry plantations is therefore recommended and, if possible, wild blackberries should be eradicated from such situations.

Special care should be taken with material used for propagation and all infected plants should be removed as soon as they are detected. It is difficult to diagnose Stunt in rooted tips unless the disease is in an advanced stage, but if the tips are grown on to one-year bedded plants, as is customary, infected plants can usually be identified without difficulty. The disease is most readily recognized in the parent plants, however, and it is therefore advisable to keep the rooted tips from each plant separate, so that they can be identified and destroyed if symptoms of Stunt appear on the parent in the spring following the removal of the tips. If tips from infected plants are grown near tips from healthy plants, the virus may spread before the infected tips can be recognized.

Similar precautions may prove effective with raspberry varieties such as Malling Landmark. At East Malling, the roguing out of all spawn within four feet of diseased canes apparently eliminated the disease, within two years, from a propagation bed of this variety. The problem of varieties in which the disease has a long incubation period is more difficult. At East Malling, however, the roguing of visibly infected

PLATE I.

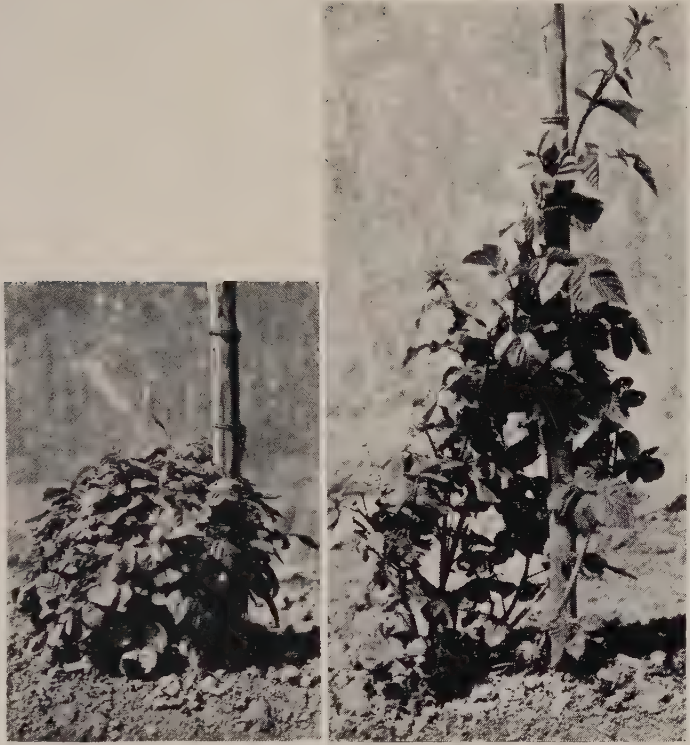

Fig. 1.

Left : Loganberry with Stunt. *Right* : Normal loganberry.
(Fruiting cane removed.)

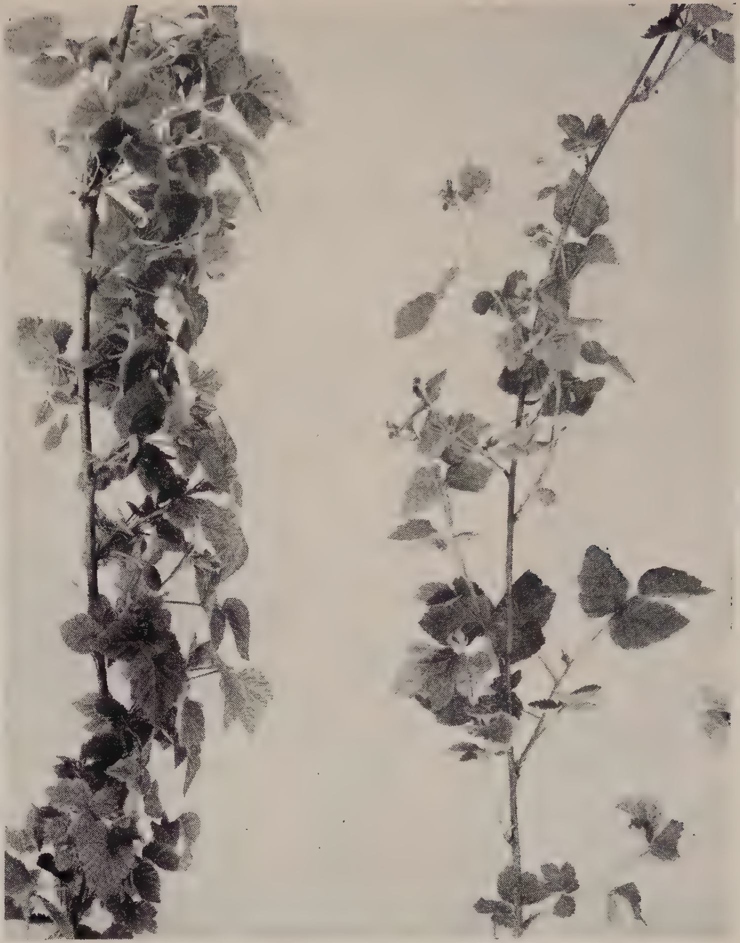

Fig. 2.

Fruiting cane of loganberry.
Left : Stunt. *Right* : Normal.

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PLATE II.

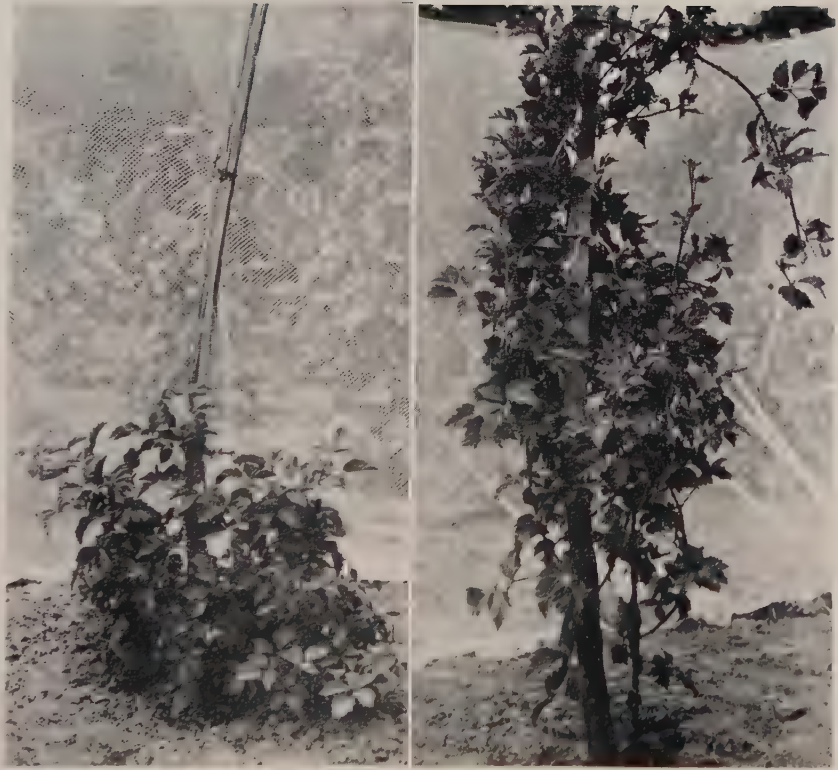

FIG. 3.

Parsley-leaved blackberry.

Left : Stunt. *Right* . Normal.

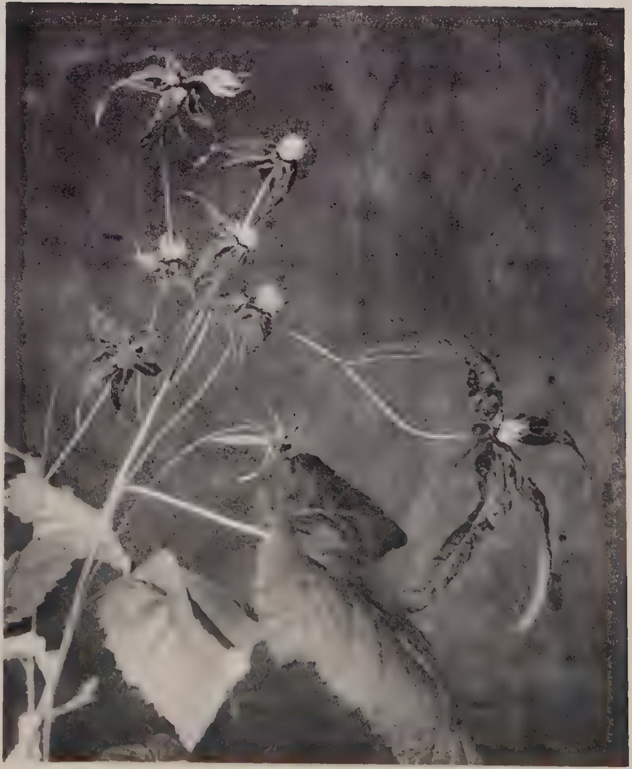

FIG. 4

Abnormal flowers on raspberry (Malling Landmark).

PLATE III.

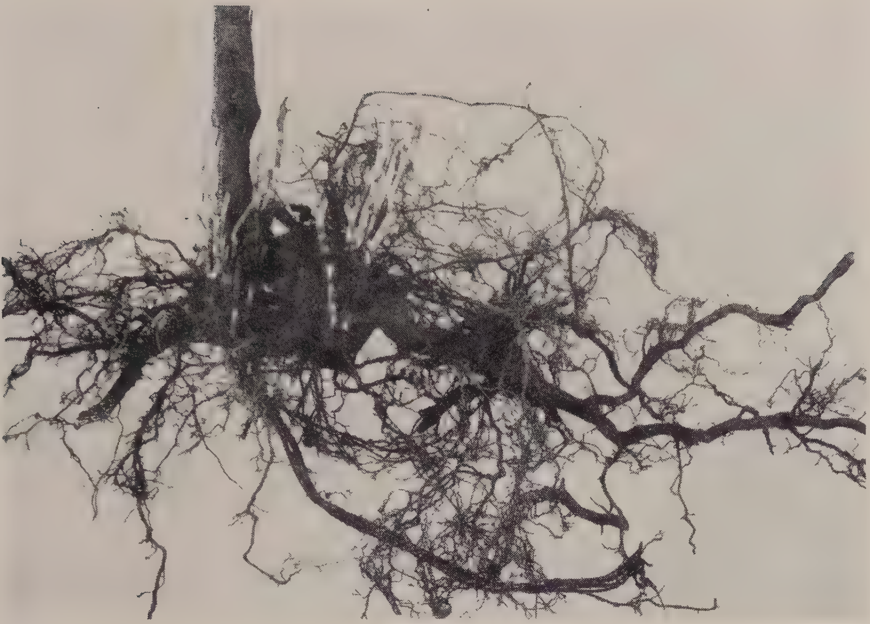

Fig. 5.
Malling Notable raspberry. Fruiting plant with Stunt.

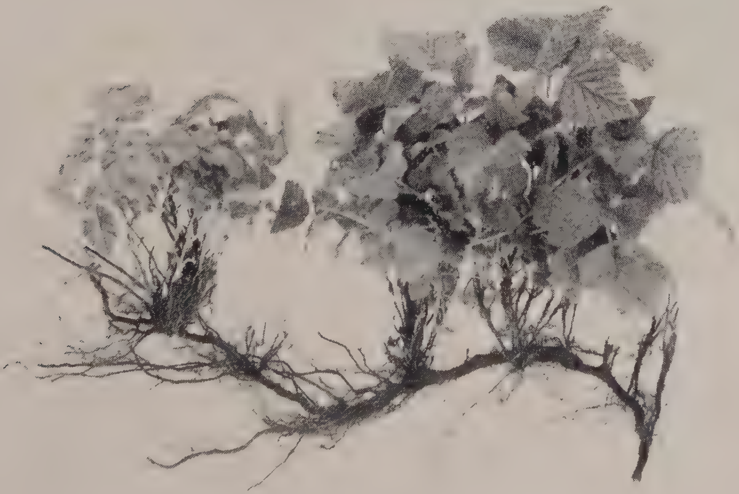

Fig. 6.
Malling Landmark raspberry. Spawn with Stunt. (From propagation bed.)

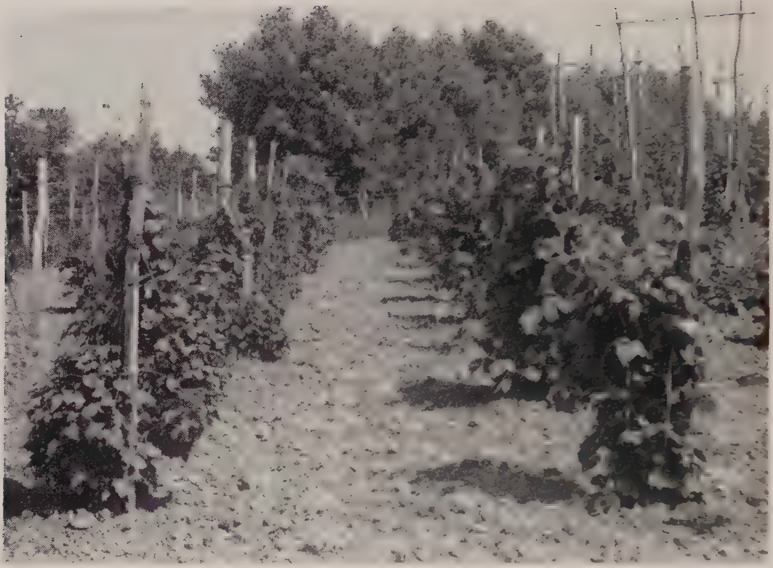

FIG. 7.
Loganberry.
Left : Five plants grafted with Stunt infectors in 1945. *Right :* Five control plants.

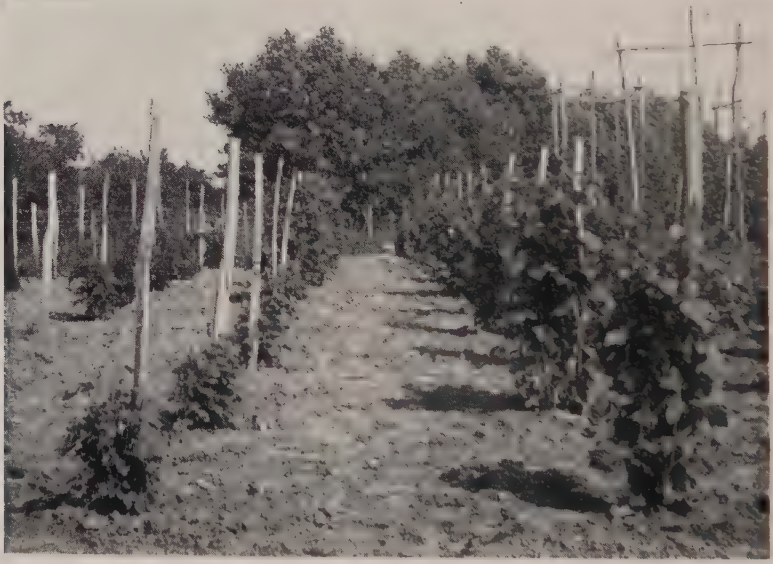

FIG. 8.
Same as Fig. 7, but with fruiting cane removed.
(Figs 7 and 8 photographed June, 1947.)

PLATE V.

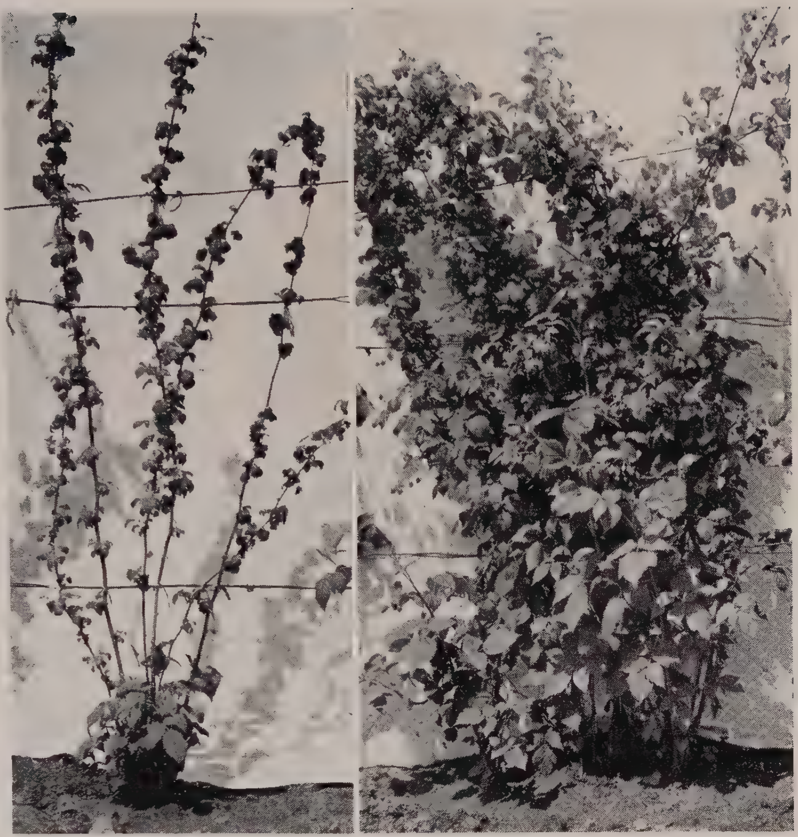

FIG. 9.
Norfolk Giant raspberry.
Left: Stunt. *Right:* Normal.
(Grafted 1945 and 1946, photographed May, 1948.)

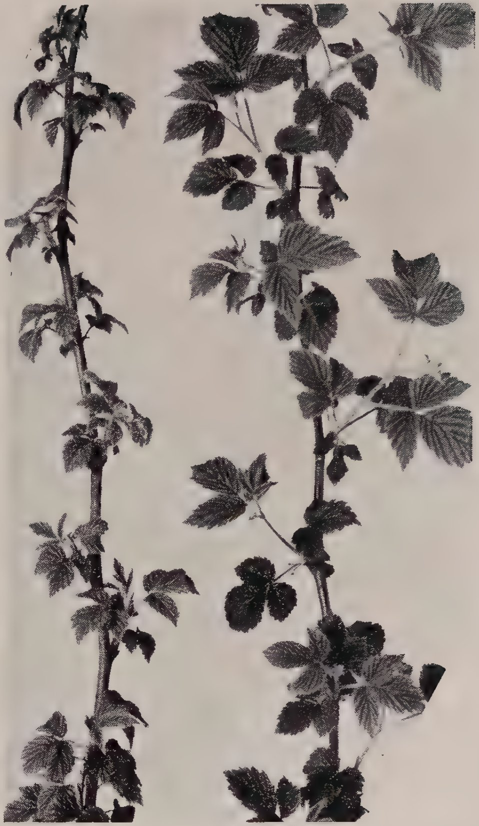

FIG. 10.
Fruiting cane of Norfolk Giant raspberry.
Left: Stunt. *Right:* Normal.

plants has, over a period of three years, considerably reduced infection in a fruiting plantation of mixed varieties, although it has not entirely eliminated it. The disease seems to spread more slowly in raspberries than in loganberries and it can probably be kept under control by careful roguing despite the unavoidable delay in identifying new infections.

DISCUSSION.

Zeller (1927) has described a dwarfing virus disease of loganberry, etc., occurring in Oregon and other Pacific Coast States of the U.S.A. The disease was practically restricted to loganberry and Phenomenal Berry, the latter being the more susceptible. It was also noted on a few occasions on certain varieties of thornless blackberry and was later reported on Boysenberry and Youngberry (Zeller and Milbrath, 1940). The first symptoms in mature plants are described as the development of spindling and flexuous canes with small leaves of abnormal shape but normal colour. Later the canes were short with short internodes and several buds at each node and the leaves became pale in colour. New canes were fewer in number each year.

In has generally been assumed that the Stunt disease on loganberry in England is the same as the Dwarf disease described by Zeller, but comparison of the symptoms as described makes this doubtful. The most characteristic feature of Stunt on loganberry is the production of numerous thin, short canes. In Loganberry Dwarf the number of canes is decreased.

Zundel (1931) reported a " Witches' broom " disease of raspberry in which the lateral branches were replaced by numerous spindle sprouts. There is no indication of an abnormal production of new cane and no evidence that the disease was transmissible. The fruiting laterals on raspberry affected with Stunt are short but not in bunches (although they are on blackberry, etc.) and the number of new canes produced is large. It is therefore considered that the diseases are probably distinct.

Plakidas (1937) has described a Rosette disease of blackberries and dewberries caused by *Cercosporella rubi*. Some or all of the lateral branches on infected canes develop " witches' brooms ". The tips of the calyx segments are leafy and sometimes the whole sepal develops into a leaf. Sometimes the petals become leaflike, the stamens sterile and the carpels leaflike. The base of the receptacle may elongate so that the pistils are carried up on a short stalk. Although some of the symptoms are very similar, this disease is obviously distinct from Stunt in that the causal fungus is readily visible on infected flowers and can be isolated and its pathogenicity demonstrated.

Recently a dwarfing disease has been briefly reported from California on loganberry, Phenomenal berry, etc. (Wilhelm *et al.*, 1948). Among the symptoms mentioned are " the precocious development of basal buds giving plants . . . a bunchy appearance ". Raspberry is thought to be a symptomless carrier, but when further details are available, this disease may prove to be related to Rubus Stunt.

SUMMARY.

Rubus Stunt is a disease of loganberry and blackberry prevalent in southern England. It also affects raspberry and other cultivated species and hybrids of *Rubus*. Infection in all hosts results in the production of very numerous weak canes, so that the infected plant has a bushy appearance. The disease, which

spreads rapidly in loganberry and less rapidly in raspberry, is transmissible by grafting and is considered to be a virus disease. The vector is not known. Control by roguing is recommended.

Rubus Stunt is considered distinct from any virus disease of *Rubus* so far described, with the possible exception of a dwarfing disease of brambles reported from California.

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(*Received* 11/2/50.)

RIPENING OF TOMATOES

II. FURTHER EXPERIMENTS ON THE EFFECTS OF ETHYLENE

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INTRODUCTION.

THE results obtained in our previous work (Fidler and Nash-Wortham, 1949) confirmed the earlier observation (Furlong, 1944) that ethylene may have little effect on the speed of ripening of fully grown green, or turning, tomatoes.

In our previous work the treatment of the tomatoes with ethylene was not continuous for the whole of the ripening period; ethylene was given for two to five days only, as is usual in the treatment of bananas and other fruits. Further, while the fruit we used (which was obtained from a commercial grower) appeared to be fairly uniform in size and maturity, it displayed great variability during ripening. In the present experiments the treatment with ethylene was continuous, and carefully selected fruit of known history was used. The treatments had no significant effect on the ripening of either indoor-grown or end-of-season outdoor tomatoes.

EXPERIMENTAL.

The indoor-grown tomatoes came from the Experimental and Research Station, Cheshunt, Herts., and were carefully selected from fruits in the middle of the truss only, showing the first traces of yellowing. They reached the laboratory and were put under experimental conditions on the day they were picked.

Fifty fruits of each variety were ripened in air and fifty in air plus ethylene (1 part of ethylene to 650 parts of air in most trials), in each experiment. The first trial (21st June), was carried out at 65° F., but tomatoes in 1949 ripened so rapidly that the experiment was virtually completed by the third day. Accordingly,

TABLE I.

Ripening of indoor-grown tomatoes in air, and in air plus ethylene, at 65° F. (Experiment began 21/6/49).

Variety.	Conditions.	Progress of ripening (percentages).			
		After 1 day.	After 2 days.	After 3 days.	After 4 days.
Ailsa Craig ..	Air	14R : 82Y	28R : 72Y	92R : 8Y	100R
	Air+ethylene (1 : 650)	18R : 60Y	22R : 72Y	100R	100R
Potentate ..	Air	18R : 54Y	68R : 6Y	78R : 22Y	100R
	Air+ethylene (1 : 650)	16R : 42Y	54R : 18Y	100R	100R

R. = Ripe. Y. = Yellow.

TABLE II.

Ripening of indoor-grown tomatoes in air, and in air plus ethylene, at 55° F.

Date experiment began.	Conditions.	Progress of Ripening.
A. Ailsa Craig: 14/7/49	Air	4 days—56Y 7 ,, —48R : 52Y 11 ,, —86R : 14Y 12 ,, —100R
	Air+ethylene (1 : 650)	4 days—52Y 7 ,, —48R : 52Y 11 ,, —90R : 10Y 12 ,, —100R
17/8/49	Air	9 days—92R : 4Y 12 ,, —96R : 2Y 13 ,, —98R : 2G
	Air+ethylene (1 : 650)	9 days—92R : 8Y 12 ,, —98R : 2Y 13 ,, —100R
20/9/49	Air	6 days—82R : 6Y 13 ,, —86R : 6Y : 8G
	Air+ethylene (1 : 400)	6 days—84R : 12Y 13 ,, —92R : 4Y : 4G
B. Potentate: 14/7/49	Air	4 days—64Y 7 ,, —50R : 50Y 11 ,, —84R : 16Y 12 ,, —100R
	Air+ethylene (1 : 650)	4 days—54Y 7 ,, —56R : 44Y 11 ,, —90R : 10Y 12 ,, —100R
17/8/49	Air	9 days—60R : 34Y 12 ,, —94R : 2Y : 4G 13 ,, —96R : 4G
	Air+ethylene (1 : 650)	9 days—76R : 10Y 12 ,, —84R : 16Y 13 ,, —100R
20/9/49	Air	6 days—84R : 6Y 13 ,, —86R : 8Y : 6G
	Air+ethylene (1 : 400)	6 days—78R : 10Y 13 ,, —86R : 14Y

R. = ripe. Y. = yellow. G. = persistent green.

TABLE III.

Ripening of outdoor-grown tomatoes in air, and in air plus ethylene.

Variety.	Date experiment began.	Temperature ° F.	Conditions.	Progress of ripening (percentages).	Notes.
Hundredfold..	4/10/49	55	Air	14 days—93R 7T (21 days—30% rots)	Final picking. All very backward initially.
			Air + ethylene (1 : 650)	14 days—89R 11T (21 days—62% rots)	
Moneymaker..	4/10/49	55	Air	14 days—73R 20T 21 days—87R 9T	Final picking. All very backward initially.
			Air + ethylene (1 : 650)	14 days—65R 33T 21 days—82R 11T	
Donas ..	25/10/49	65	Air	6 days—33R 9 days—78R 14T 5 rots	From strippings of plants. Dead green.
			Air + ethylene (1 : 650)	6 days—38R 9 days—77R 16T 7 rots	

R. = ripe. T. = turning.

subsequent experiments were done at 55° F., at which temperature tomatoes are known to ripen normally.

The outdoor tomatoes came from the same market garden at Swanley, Kent, as those used in the previous year. Being end-of-season fruit, their maturity was variable. They were put under experimental conditions on the day after picking.

In all experiments, the relative humidity of the ripening chambers was kept high by putting a tray of water under the trays of fruit.

The results are given in Tables I, II and III.

DISCUSSION.

The tables are self explanatory, and present no difficulties in interpretation. It is clear that the two samples of any one variety, in any experiment, behaved in essentially the same manner. The speed of ripening was similar, in air alone or in air containing ethylene.

The glasshouse fruit contained a few individuals which showed no sign of ripening at all. These were in all cases the smallest fruits in the samples; they may have been immature.

The data confirm our previous conclusion that mature tomatoes may usually be ripened satisfactorily in a warm room with a fairly high relative humidity, and that the use of ethylene is not worth while.

SUMMARY.

Ethylene does not significantly accelerate the ripening of indoor or end-of-season outdoor-grown tomatoes.

ACKNOWLEDGMENTS.

We wish to thank Dr. W. F. Bewley, C.B.E., Director of the Cheshunt Research Station, for supplying the tomatoes.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research, and is published by permission of the Director.

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(*Received* 18/3/50.)

STRAWBERRY CULTIVATION STUDIES

III. SPACED AND MATTED SYSTEMS

By W. S. ROGERS and IRENA MODLIBOWSKA

Introduction.

In choosing a cultural system for strawberries, as for other commercial plants, two main groups of factors, which may broadly be classed as ecological and economic, need to be considered.

The ecological factors include arranging the plants so that their roots and leaves are in the best possible environment, to enable them to make the best use of the available nutrients, water and sunshine, and thus produce good plants and fruit. The economic factors include provision for convenient and efficient cultivation and handling of the crop, to suit the labour and machinery available.

The initial planting distance and arrangement can be important from both points of view; and, in the strawberry, the natural production of runner plants, which will root near the parents if allowed to do so, provides a further range of possible cultural systems.

There are thus two main systems of growing strawberry plants for fruit production.

1. The *single plant system*, in which all the runners are removed and only the original plants are left to fruit.
2. The *matted system*, in which some or all runners are allowed to grow round the original plants, forming continuous rows or beds.

Many modifications of these systems are possible, and consist mainly in variations of spacing of rows and plants, and restriction of number of runners in the matted systems.

Practical experience has provided some information on the merits and demerits of the various systems, and many advisory leaflets contain general comments upon them, but few experimental comparisons have been made either in England or abroad.

While both single plant and matted systems are used in England, single plant systems are the most common. More attention has been given to matted systems in the U.S.A. and Canada. Schrader & Haut (1936), in an experiment under dry conditions in Maryland, found that matted beds produced less and smaller fruit than spaced beds. This also held, but to a less extent, when irrigation was applied; but it was noted that the crowded plants had probably suffered to some extent before irrigation was begun. Peacock (1939), on the other hand, in experiments on thinning of runners from matted beds, in Maryland, found that the total yield of fruit was usually reduced in proportion to the severity of thinning, although overcrowding of plants increased the percentage of small fruits.

Minges *et al.* (1942), in Iowa, recommend moderate spacing and thinning depending upon the variety.

Advisory bulletins, describing recommended commercial practices in various parts of the U.S.A. and Canada include those of Waldo & Hartman (1947), French (1945), and Davis & Blair (1945).

A series of experiments comparing different spacing of plants and, later, spaced and matted systems, was carried out at East Malling from 1935 onwards and the first three of these are reported below:

TRIAL I.

The effect of distance of planting in the single plant systems.

Three distances of planting, 9 in., 18 in. and 36 in., in rows uniformly 36 in. apart, were compared. The experimental plots were 8 yds. long and 4 rows wide, thus containing 128 plants at 9 in., 64 at 18 in., or 32 at 36 in., respectively. There were guard rows similarly spaced at each side of each plot, and guard plants at the end of each experimental row. There were 16 replicate plots of each treatment. A manurial experiment which was superimposed on the spacing trial will be reported elsewhere. No differential effects of manuring and spacing were found in the first two years, but in the third year the benefit of manuring was more pronounced in the crowded plots than in the wide spaced ones.

The plants were of the variety Royal Sovereign (Malling 35 stock) and were planted in October 1935, under good conditions.

Rainfall. The total rainfall for 1936, 1937 and 1938 respectively was 28·6, 33·5 and 23·0 in., compared with a 30 years average of 26·2. Thus 1937 was a very wet year, and 1938 a dry one. The same contrast is seen in the critical month of June when rainfall was 2·30 in. in 1937 and 0·58 in. in 1938, compared with a 30 years average of 1·44. This is of importance in relation to the plant competition effects discussed later.

Plant size. The heights and spreads (across the row) of plants were measured periodically, with special measures marked in inches (Rogers, 1931.). The results are shown in Tables I and II. No important differences appeared by the first spring (1936) but, by September, the 9 in. spaced plants were taller than the others, probably as a result of the "drawing up" effect of the close planting (Table I). There were no significant differences in spread across the row; but the plants at 9 in. had already begun to compete by September, 1936, for their width was over 18 in., and their diameter along the row could not be more than 9 in. without touching.

TABLE I.

Heights in inches of strawberry plants at different spacings.

Date	Spacing in row 9 inch.	18 inch.	36 inch.	Significant difference (P=0·05)
May, 1936	4·3	4·1	4·3	Not significant
September, 1936 ..	8·8	7·9	7·8	0·38
May, 1937	9·9	9·2	9·5	0·54
October, 1937	5·5	5·8	6·7	0·47
July, 1938	8·2	8·6	9·1	0·45

TABLE II.

Spreads of strawberry plants at different spacings.

Date.	Spacing in row 9 inch.	18 inch.	36 inch.	Significant difference (P=0·05)
May, 1936	8·3	8·3	8·2	Not significant
September, 1936	18·4	18·3	18·5	,,
May, 1937	17·7	18·6	19·1	0·81
October, 1937	17·2	17·3	17·8	Not significant
July, 1938	18·3	20·2	20·2	0·90

In spring 1937, the competition between closely spaced plants became more pronounced, as shown by the spreads (Table II). The closest plants were still the tallest, but the difference was small and not significant. Both in October, 1937, and July, 1938, the widest spaced plants were the largest, in height and spread, but the increase in size was not nearly proportional to the extra room available to them, i.e. at no time were the plants at 36 in. four times as big as those at 9 in.

Runner production. The runners produced by the plants were cut off periodically, so as to leave only the parent plants. Weights of runners were recorded in 1936 and 1937, and serve as another measure of the vigour of the plant. Table III shows that the competition effect was beginning to show by July, 1936, and continued very markedly thereafter. The contrasts were, in fact, larger in runner production than in plant size. The 9 in. spaced plants produced rather less than half the amount of runners per plant of the 36 in. spaced ones. This is in line with more detailed subsequent work on runner production (Rogers, 1947), which showed that, within limits, the wider the spacing of parent plants, the greater the amount of runners *per plant* and the closer the spacing, the greater the amount of runners *per acre.*

TABLE III.

Production of runners: gm. per plant.

Date	Spacing in row 9 inch.	18 inch.	36 inch.
June, 1936	7·4	6·7	6·8
July, 1936	35·5	39·0	45·7
September, 1936	66·6	91·3	101·2
Total 1936	109·5	137·0	153·7
Total 1937	27·2	45·4	63·6

Blossom production. Deblossoming was carried out as a routine operation in the first spring, 1936, in order to invigorate the plants. The blossom truss weights, *per plant*, are shown in Table IV(i). These show no significant differences between

TABLE IV.

Effect of spacing on yield.

Date.		Spacing in row 9 inch.	 18 inch.	 36 inch.	Significant difference (P=0·05)
(i) 1936	Weight of blossom trusses gm. per plant	1·67	1·54	1·58	Not significant
(ii) 1937	Crop. Tons *per acre*	4·58	3·57	2·22	0·16
	lb. *per plant*	0·52	0·81	1·01	
1938	Crop. Tons *per acre*	2·13	2·02	1·26	0·19
	lb. *per plant*	0·24	0·46	0·57	

the spacings. Thus, had the plants been allowed to crop, the 9 in. spaced plots would doubtless have carried much the heaviest crop, *per plot*, because they had the most plants.

Fruit yield. The cropping figures for the second and third years after planting, 1937 and 1938 (Table IV(ii)) are of great interest. They show that, within these limits, the closer the spacing the larger the crop *per acre*, and the smaller the crop *per plant*. The increase is not directly proportional, but a fourfold increase in number of plants resulted, in 1937, in doubling the crop per acre. In 1938 the amount of crop was reduced by spring frosts. A survey, made by counting all the damaged and undamaged blossoms on sample plants (four plants chosen at random from each plot), showed that about 35 per cent. of the blossoms had been damaged, and there were no significant differences between the differently spaced plants.

Added to this, the very dry weather in June doubtless lessened the yield. Nevertheless, as Table IV shows, quite a good crop was picked. It can be seen that, once more, the plots at 18 in. had a 50 per cent. greater crop than those at 36 in., but those at 9 in. had no greater crop than those at 18 in. This suggests that the severe plant competition at 9 in., under these dry conditions, had become a limiting factor. On the other hand, the plants at 36 in. evidently could not fully utilize the extra space allotted to them.

Size of fruits. The fruits were all picked in three grades, as follows :

Grade 1. Fruits within the " Extra selected " and " Selected " grades of the Ministry of Agriculture (minimum weight approximately ½ oz.).
Grade 2. Good fruits of smaller size.
Grade 3. Rotten and damaged fruits.

In 1937, the good moisture conditions are reflected in the very high percentage of fruit in Grade 1 (Table V(i)) and the lack of any significant difference between the size of fruits from differently spaced plants. In 1938, the wider spaced plants had a higher percentage of first grade fruit. The percentage of first grade fruit was also smaller than in 1937, in spite of a smaller crop. This is doubtless due to the dry conditions, as well as to the fact that the plants were a year older.

Time of ripening of fruit. The earliness of ripening of the fruit, as shown by the percentage of the total crop gathered in the first two pickings, was not significantly affected by the spacing, in this experiment.

TABLE V.

Effect of spacing on fruit quality.

	Date.	Spacing in row 9 inch.	18 inch.	36 inch.	Significant difference (P=0·05)
(i) Percentage of crop in Grade I	1937	72·7	72·8	74·9	Not significant
	1938	27·9	30·1	33·6	3·76
(ii) Percentage of rotten and damaged fruit	1937	19·3	16·6	13·1	2·95
	1938	9·1	7·6	7·3	Not significant

Rotting of fruit. In the wet picking season of 1937, rotting of the fruit by *Botrytis* was severe. This is reflected in the high amount of fruit in Grade 3. As Table V(ii) shows, the plants at 36 in. had a significantly smaller amount of rotten fruit than those at 18 in. The plants at 9 in. had more rotten fruit than those at 18 in., but the difference is not significant.

In the dry season of 1938 the amount of rotten fruit was much less, and showed no significant differences between the different spacings.

When the severity of rotting in 1937 was observed, an attempt was made to lessen it by bunching up the leaves with 3 in. by $\frac{1}{16}$ in. rubber bands, on half the plots. This operation did not prove successful in reducing the percentage of rotten fruit, however. Possibly it was too late to be effective.

Conclusions. This trial showed clearly that spacing of Royal Sovereign plants as much as 36 in. square resulted in a considerable reduction in crop per acre, both in wet and dry years, when compared with the more normal spacing of 18 in. by 36 in. Still closer spacing, at 9 in. by 36 in., gave a considerable increase in crop, compared with 18 in. by 36 in., in a wet year, but no increase in a dry year. Eighteen inch spacing in the row, for Royal Sovereign, is desirable for experimental plots where plants need to be maintained as separate individuals, for purposes of size and health records ; but where maximum crop is the chief requirement, closer spacing is clearly justified, under soil and climatic conditions comparable with East Malling.

These findings led to a renewed interest in the matted row and bed, and when it became possible to do so, in autumn 1944, a comparative trial was started.

TRIAL II.

Single plant and matted systems.

A spaced single plant system and three matted systems, all based on an initial planting at 18 in. by 36 in. were compared, as follows :

(*a*) The normal spaced single row system, in which plants were set at 18 in. by 36 in. and all runners were cut off, leaving only the original plants.

(*b*) The matted row system, in which runners were allowed to root alongside the parent plants, to form a matted row 18 in. wide.

(*c*) The matted bed system, in which all runners were allowed to root between pairs of rows of parent plants, leaving the alternate alleys clear for cultivation.

(*d*) A spaced matted bed system, differing from (*c*) only in that from July onwards the runners were spaced to about 5 or 6 in., i.e. to 42 plants per square yard.

The experimental plots were each 9 yds. long and 2 yds. wide, thus initially containing 36 parent plants. The treatments were distributed at random in six blocks, three of which were planted entirely with first grade plants and the other three with second grade, smaller plants of Royal Sovereign M.40. There was no difference in growth and cropping of the plants of initially different grades, from the first summer onwards, as was also found in earlier experiments (Rogers & Edgar, 1938). This comparison therefore needs no further consideration in the present paper.

Rainfall. The total rainfall for 1946 was 32·43 in. and that for 1947 23·72 in., i.e. 1946 was a wet year and 1947 a dry one. The rainfall for the month of June was above the average in both years, being 1·68 in. and 2·17 in. respectively, but in 1947 much of the rain came at the end of the month. During the picking season of 1946 soil conditions were moist, the soil moisture tension being below 20 cm. Hg almost the whole time, while in 1947 it was above 30 cm. Hg for much of the time, and rose to a maximum of 60 cm. Hg.

Plant Growth. In the first year after planting all the plants were deblossomed, and the runners were either cut off or trained into different systems as described above.

The methods are illustrated in Fig. 3.

Plant Vigour. The vigour of the original plants was recorded by measuring their heights.

TABLE VI.

Heights in inches of strawberry plants grown under different systems.

Date.	Cultural System. Spaced single row.	Matted row.	Matted bed.	Spaced matted bed.	Significant difference (P=0·05)
May, 1946 ..	6·86	7·42	5·97	6·03	0·70
October, 1946 ..	5·85	6·57	5·03	4·64	0·34
July, 1947 ..	7·95	9·59	8·94	8·08	0·61

Table VI shows that, two years after planting, i.e. when the different systems were established and cropping, the plants in the matted row system were significantly taller than those of any other system. This is probably due to a drawing-up effect of the runners surrounding the parent plant similar to that observed in the close planted plants in Trial I.

The plants of system (*a*), the single spaced row, were obviously more spreading than those of the matted systems, but it was not practicable to measure the spreads of individual plants in the matted systems because of the intermingling of the leaves.

Fruit yield. Very striking effects of the different systems were shown in the fruit yields, as shown in the first part of Table VII. The yield of the matted beds reached 6 tons per acre, twice that of the spaced single rows, in 1946, and the matted rows reached 5·5 tons per acre. In 1947 the yields were smaller, probably owing to the drier conditions, but all the matted systems again exceeded 4 tons per acre,

TABLE VII.

Amount and quality of crop under different systems.

		CULTURAL SYSTEM.				
	Date.	A Spaced single row.	B Matted row.	C Matted bed.	D Spaced matted bed.	Significant difference (P=0·05)
(i) Marketable crop. Tons per acre ..	1946 1947	3·0 2·5	5·5 4·2	6·4 4·4	6·0 4·1	0·62 0·36
(ii) Percentage of crop in Grade I ..	1946 1947	60 25	64 32	59 25	65 29	not sig. not sig.
(iii) Percentage of rotten and damaged fruit	1946 1947	14 5	13 7	11 6	12 5	not sig. not sig.
(iv) Percentage of total crop in first 2 picks	1946 1947	6·2 26·6	4·6 21·6	2·6 23·3	10·2 33·6	3·33 6·83

a yield 60 per cent. greater than that of the single row. The reduction from the previous year was greatest in the matted beds and least in the single row, as might be expected under drier conditions.

Fruit size. The second part of Table VII shows that the size of fruit, as shown by percentage of Grade I fruits, did not differ significantly between the systems, a rather surprising result, especially in the second and drier season. The general reduction in size in the drier year is clear.

Fruit rotting. The third part of Table VII shows that there was no difference between the systems in percentage of fruit that was rotten or damaged. The general reduction in rotting in the drier year is evident, however. It is especially noteworthy that in a relatively wet picking season such as 1946, even the matted beds had no greater proportion of rotten fruits than the single rows.

Earliness of fruiting. Part (iv) of Table VII shows that the spaced matted bed ripened its fruits slightly earlier than the other systems, in both seasons. The explanation is not quite clear, for if it is due to earlier ripening of fruit from maiden plants (the runners) one would expect it also to show in the matted row and unspaced matted bed. It may be, however, that in the unspaced matted bed the shading caused by the denser foliage delayed ripening, and in the matted row the proportion of young to older plants was smaller.

TRIAL III.

Comparison of six systems.

In this experiment six systems were compared, as follows:

(1) The spaced single row, at 18 in. by 36 in., as in Trial II (Fig. 1(*a*)).

(2) A triple row system, using the first runner only, from each plant. The runners are laid in the middle of alternate alleys, forming new rows with plants 9 in. apart (Fig. 1 (*b*)). This system is used to some extent in the Clyde Valley of Scotland.

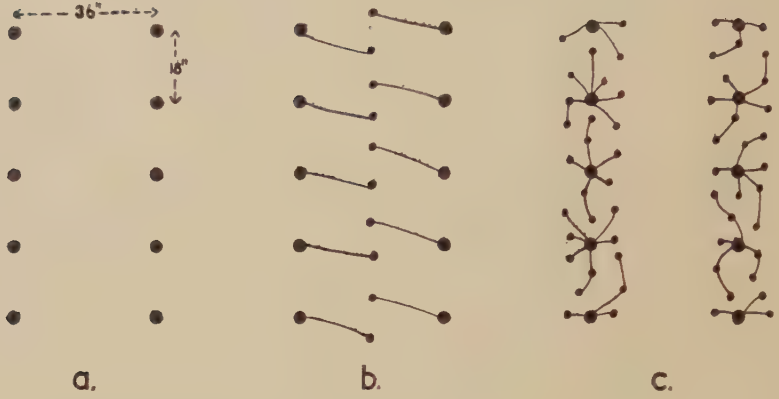

FIG. 1.
(*a*) Single spaced row; (*b*) triple row; (*c*) spaced matted row.

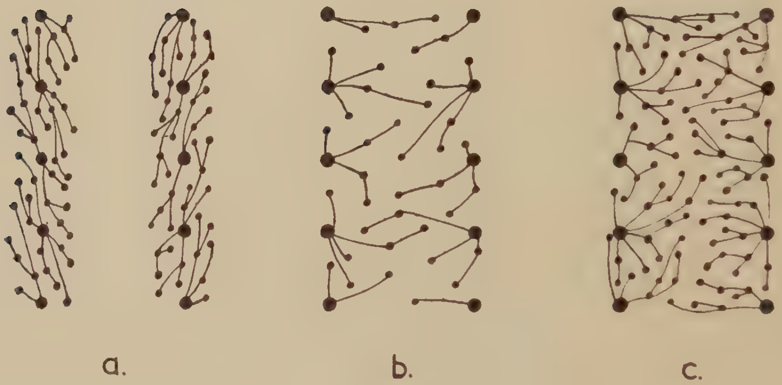

FIG. 2.
(*a*) Unspaced matted row, runners swept in by hoeing; (*b*) spaced matted bed; (*c*) unspaced matted bed.

(3) A spaced matted row, in which about 7 runners per plant are laid in the row about 7 in. apart, to a width of about 18 in. (Fig. 1 (*c*)).

(4) An unspaced matted row (Fig. 2 (*a*)) in which all runners were swept in by hoeing, instead of being laid in as in Trial II. The object was to reduce hand labour.

(5) A spaced matted bed, having 20 runners laid in every square yard, between two rows, allowing a spacing of 7 to 8 in. (Fig. 2 (*b*)).

(6) An unspaced matted bed, as in Trial II (Fig. 2 (*c*)).

There were four replicate plots of each treatment, each initially containing 2 rows of 18 plants of Royal Sovereign M.40, planted at 18 in. by 36 in., in November, 1945.

Plant growth. As in Trial II, the plants grown singly clearly had the largest spread, and the plants of the unspaced matted row were the tallest in 1946 (Table VIII), but the following year they were slightly smaller than those of all treatments except the unspaced matted bed. Evidently plant competition was then becoming severe. The runners themselves showed conspicuous differences. In autumn of the first year the limited runners of the triple row were, as might be expected, the largest. Runners in spaced matted rows and beds had larger and darker leaves than those in the unspaced ones, which appeared overcrowded and showed more autumn coloration.

TABLE VIII.

Heights in inches of plants grown under different systems.

	CULTURAL SYSTEM.					
Date.	Spaced single row.	Triple row.	Spaced matted row.	Unspaced matted row.	Spaced matted bed.	Unspaced matted bed.
October, 1946 ..	4·35	4·25	4·30	5·40	4·00	4·45
July, 1947	10·00	10·20	10·70	9·95	10·00	8·90

Fruit yield and quality. Table IX shows that, as in Trial II, fruit yield was greatly affected by the cultural system. In every case the matted systems gave a significantly higher crop than the single row, the increases ranging from about 43 to 52 per cent. for matted rows, and 65 to 91 per cent. for matted beds. The triple row gave a 56 per cent. increase. The spaced systems gave higher crops than the unspaced ones which, in this case, appeared definitely overcrowded (Fig. 4).

This is borne out by the fact that the proportion of Grade I fruits, as well as the yield, was significantly smaller in the unspaced than in the spaced matted beds, in fact, smaller than on any other plot (Table IX (ii)). Nevertheless, the actual *amount* of Grade I fruits, even on the unspaced matted beds, was as great as that on the single plant rows, on account of the higher crop of the former.

It is rather curious that the percentage of rotten and damaged fruit was significantly higher on the single plants than in the matted rows, in this experiment, in a relatively dry year.

TABLE IX.

Amount and quality of crop, 1947.

	CULTURAL SYSTEM.						
	Spaced single row.	Triple row.	Spaced matted row.	Un-spaced matted row.	Spaced matted bed.	Un-spaced matted bed.	Significant difference (P=0·05)
(i) Marketable crop. Tons per acre ..	2·3	3·6	3·5	3·3	4·4	3·8	0·31
(ii) Percentage of crop in Grade I ..	39	38	37	35	35	24	8·10
(iii) Percentage of rotten and damaged fruit ..	11·0	8·0	8·4	7·6	6·2	6·0	2·62
(iv) Percentage of total crop in first 2 picks..	9·2	11·1	14·1	8·7	17·8	11·7	6·37

The fruit on spaced matted beds and rows was again slightly earlier than that on other systems (Table IX (iv)), but the only differences that were significant were those between spaced matted beds and single rows or matted rows.

Strawing. Strawing of matted rows and beds was only possible in the spaces between them, but the fruits on these crowded plants usually did not become splashed with soil. In the triple row the plants were too close for strawing but not close enough to prevent splashing, so the risk of damage was increased.

Diseases and pests. Any system involving very close growing of plants tends to increase the risk of rapid spread of diseases and pests. This was not shown in our experiments, possibly because the plots were small and randomized. The matted systems make the elimination of infected plants very difficult however. Trial III had to be discontinued after the 1947 crop was picked, on account of spread of the strawberry tarsonemid mite.

DISCUSSION.

These experiments have shown clearly that, within the limits tested, increasing the number of plants per acre has increased the crop of fruit, generally without lowering of quality.

Wide spaced single rows evidently do not lead to most efficient use of the total available soil moisture and nutrients by the roots, nor of radiation by the leaves, under the conditions tested. Wide spacing has its uses for certain purposes, as in research experiments where the plants must be kept as individuals for recording purposes. Again, wide spacing, especially planting on the square to enable mechanical cultivation in both directions, can result in great saving of labour, but only at the cost of lower yield per acre.

These experiments suggest that, in strawberries, root competition becomes a limiting factor before leaf competition, for cropping was not reduced in matted

STRAWBERRY CULTIVATION STUDIES. III. SPACED AND MATTED SYSTEMS. BY W. S. ROGERS AND IRENA MODLIBOWSKA.

PLATE I.

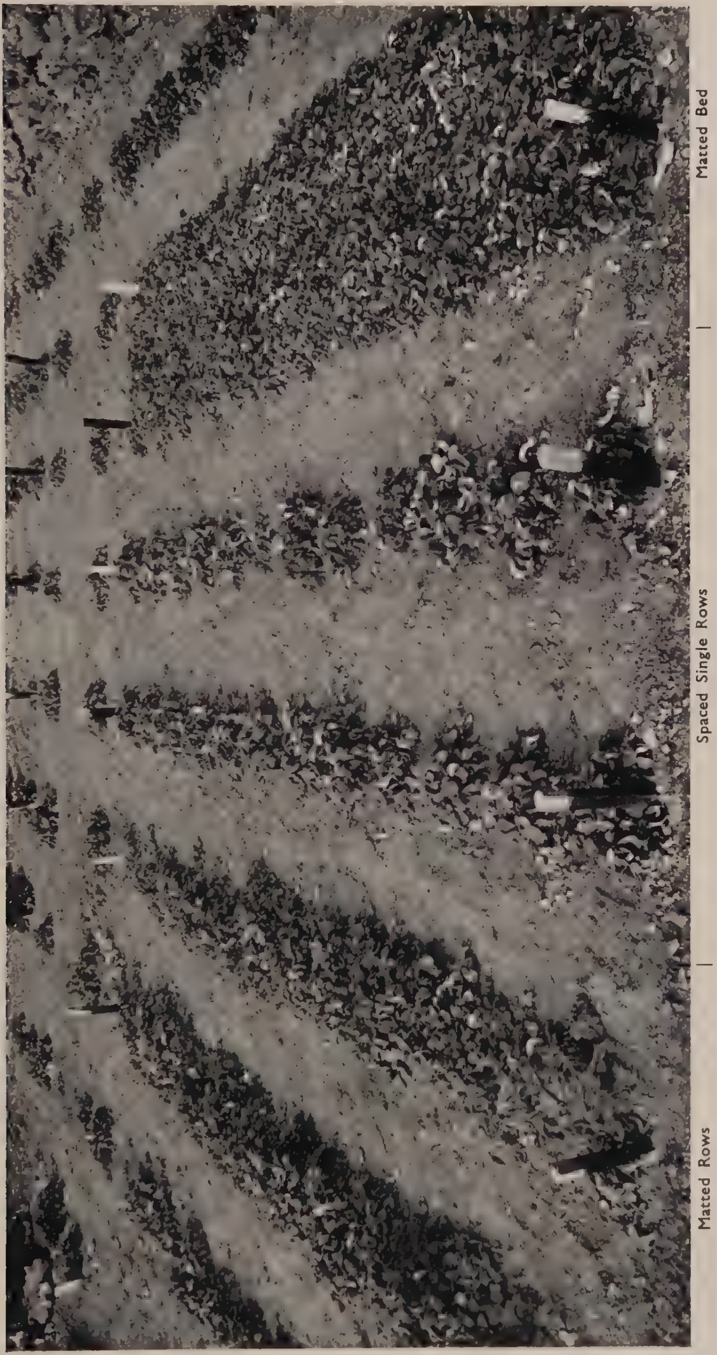
Matted Rows
Spaced Single Rows
Matted Bed

FIG. 3.
Three systems of growing strawberry plants:
(1) Matted row. (2) Spaced single row. (3) Matted bed.

PLATE II.

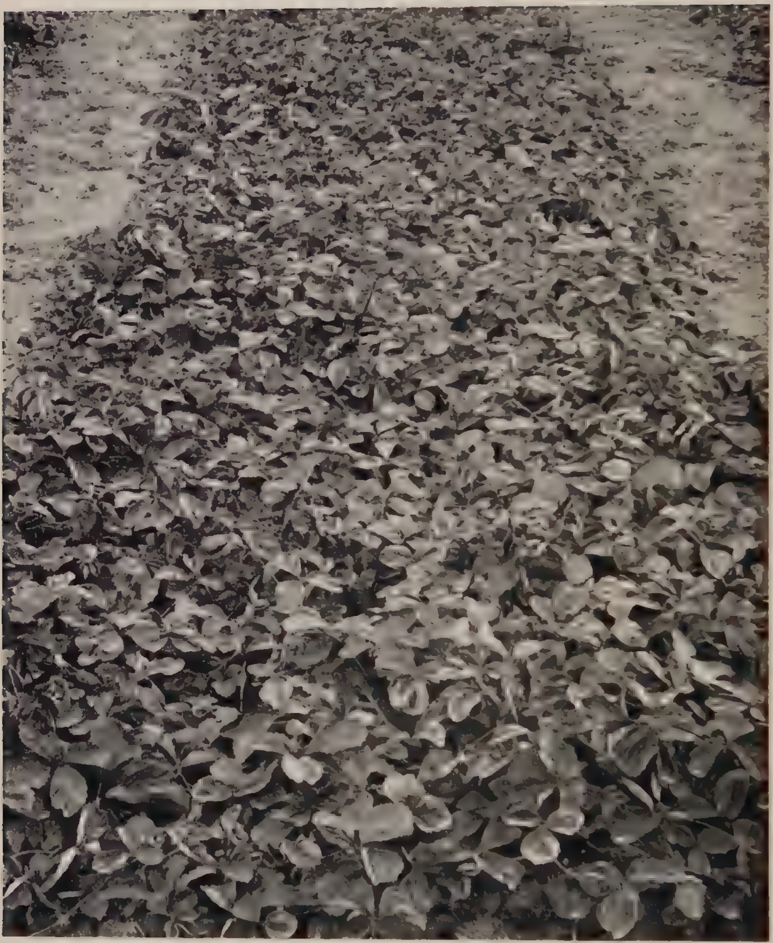

FIG. 4.
Two-year-old matted bed showing overcrowding.

beds, in wet seasons, in spite of undoubted crowding of the leaves ; but in dry seasons, even though the radiation was naturally greater during the longer cloudless periods, fruit size suffered. This agrees with the finding that spacing of plants, in matted systems, has in general proved desirable in the drier strawberry areas of the U.S.A. (Schrader and Haut, 1936 ; Minges, *et al.*, 1942).

Though little or no wilting was observed in the matted beds in the present series of experiments, in some other years severe wilting was seen, so it is clear that, even under Malling conditions, the matted bed probably runs a great risk of suffering from drought.

It is fortunate that the leaves need less room than the roots, for it is clearly impracticable for the leaves to occupy the whole ground area of a field, on account of the need for ready access for picking, weeding and other operations. But the roots can be allowed to fill the whole soil block, below the level of cultivation. This point lends further support to the common observation that shallow rather than deep cultivation is likely to be most beneficial for strawberries, under normal conditions.

Having established these principles, we can consider which method is likely to satisfy the practical requirements best.

Close planting within the row, though it can result in a good leaf area and a good root run to either side, and will thus give heavy cropping, is expensive to plant in the first place. It would probably be worth consideration only if the plants were to be allowed to crop as maidens (i.e. in the first year after planting) when it should result in a fair crop of high quality fruit, followed in later years by crops a little, but not much, greater than those of an 18 in. spaced row.

The matted systems seem to present great advantages to commercial growers. The initial number of plants can be fairly low, certainly not more than 9,500 per acre as used in these trials and possibly much less, though further tests are needed to establish the minimum number.

The matted bed system, though offering the highest crops under some conditions, is probably less desirable for the commerical grower than the matted row, because the matted bed uses more hand labour for runner laying and spacing, and for weeding ; and it gives more intense root competition in dry seasons. Picking is also more difficult.

For the matted row, it is desirable to keep hand labour to a minimum by laying in only the first few runners, and restricting the row width by a disc-coulter attached to the side of the cultivating implement used. A sharp hand hoe can, of course, also be used, as was done on the small experimental plots. Limitation and spacing of the plants in either matted rows or beds should be started early in the season, for leaving it until the autumn results in weakening the plants (French, 1945 ; Mecartney, 1946; Rogers, unpublished).

The aim should be to give a full cover of leaves to the ground occupied so as to stop splashing of the fruit and to lessen the risk of weed growth, without causing excessively thick growth. With rows of Royal Sovereign, initially planted 3 ft. apart, and allowed to form a mat 15 to 18 in. wide, this probably means about 18 plants per yard of row.

The cultivated alley must, of course, be wide enough for horse or mechanical cultivation, and a 20 in. clear alley is about the minimum that it is possible to pick from without damaging the fruit. It may be that a compromise between the matted bed and matted row of the present experiments, such as alternate beds and alleys each 24 in. wide, may prove a still better arrangement, but this should be a matter for test.

The initial spacing and thickness of the final bed should depend also on the variety and the soil. Huxley would be likely to need more space than Sovereign.

As has already been said, the matted systems naturally favour spread of diseases and pests, and make field roguing more difficult. It is therefore an essential part of any matted system that the healthiest possible plants, preferably Special Stock plants, should be planted in the first place, and effective routine pest and disease control measures should be maintained.

In conclusion, it is pleasant once more to be able to recommend methods already used by some good farmers. The matted system is not, of course, new, but these comparative experiments have established some of the basic scientific principles underlying its success, and further work may indicate yet more efficient ways of relating the strawberry plant to its environment.

SUMMARY.

1. Three experiments comparing different spacings and various matted systems for growing Royal Sovereign strawberry are described.

2. It is shown that planting at 36 in. square gave a lower crop per acre than 18 in. by 36 in. In a wet year planting at 9 in. by 36 in. gave a greater crop than 18 in. by 36 in. but in a dry year there was no increase.

3. In a dry year the wider spaced plants gave a higher percentage of large fruits. In a wet year there was a larger percentage of rotten fruit from the closer spaced plants.

4. In both wet and dry seasons matted rows gave about 40 to 80 per cent. increase of crop, and matted rows up to 100 per cent. increase over single spaced rows. These matted systems showed no significant differences from spaced rows in either percentage of large fruits or percentage of rotten fruit.

5. In the third trial spaced matted rows and beds gave higher yields than unspaced matted rows and beds, and a triple row system cropped as well as the matted rows, but required more labour.

6. It is concluded that, provided good weed control is maintained, matted rows are desirable for commercial culture. Matted beds, 36 in. wide, run the risk of serious drought damage. Tests of modified matted beds are suggested.

ACKNOWLEDGMENTS.

The authors acknowledge with thanks the assistance of Dr. Th. Raptopoulos and several other members of the Pomology Section, in conducting these trials. They would also thank members of the Statistical Section for analysis of the results, and Miss D. J. Wilson for help in preparing this paper for publication.

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(*Received* 20/3/50.)

STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES

II. SYNTHETIC COMPOUNDS INDUCING MORPHOGENIC RESPONSES IN THE TOMATO PLANT

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SYNOPSIS

Certain growth-regulating chemicals when applied in small quantities to plants are well-known to influence subsequent growth. In the experiments described in this paper, over thirty substances have been applied to tomato plants and the abnormalities in growth and development arising from these treatments are recorded. The compound α-(2-naphthoxy)-phenylacetic acid (NPA) produced a variety of effects, one of which was the appearance of flower trusses in terminal positions on the plant. This abnormality has previously only been recorded in plants treated with 2 : 3 : 5-triiodobenzoic acid (TIB). Comparative experiments have shown TIB to be more active than NPA in this respect.

INTRODUCTION.

MANY workers have shown that formative effects may be induced in plants following the application of synthetic growth-regulating substances. Thus, Hitchcock, Zimmerman and their co-workers (24, 25, 26, 29) found that a large number of compounds of the phenoxy and naphthoxy aliphatic acid type may induce modifications in leaf development and general growth. 2-naphthoxyacetic acid (1, 24), for example, when applied to a number of plants including the tomato, causes a modification in form and venation of the young and immature leaves, while the older leaves show epinasty and swelling of the treated parts. Associated with these effects there may be swelling, bending and proliferation of the stem tissues with the resulting initiation of many root primordia which break through the epidermal layer. Other compounds, such as 4-chlorophenoxyacetic acid and 2 : 4-dichlorophenoxyacetic acid and various xylenoxyacetic acids are well known to induce symptoms similar to those produced by certain virus diseases in tomatoes* (11, 21, 29). A number of substituted benzoic acids, applied at low concentrations, though not causing cell enlargement to any extent or initiating the production of adventitious roots, resemble the aryloxyaliphatic acids in their capacity to modify the growth of plants (25). 2-chloro-3 : 5-diiodo-, 2-chloro-3 : 5-dibromo- and 2 : 5-dichlorobenzoic acids (12) when applied to the soil of potted tomato plants, are apparently translocated to the growing point, causing curling, frenching and malformations in the apical leaves.

2 : 3 : 5-triiodobenzoic acid (TIB) has been shown by Hitchcock and Zimmerman (26, 28) to induce somewhat unique responses in the tomato plant. In untreated plants, the flower clusters, though initiated terminally, are displaced during growth to a lateral position ; development of the branching system being strictly sympodial. In plants treated with TIB, inflorescences are again initiated terminally but remain

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* This effect is sometimes described as "frenching".

terminal during subsequent growth, so that further vegetative development is carried on by the lateral growing point adjacent to the terminal inflorescence. Side shoots are similarly affected, and may terminate directly in flower clusters, or after the production of one or two much reduced leaves. Further morphogenic effects induced by TIB are the production of abnormal swellings on the flowering axis, the development of fasciated leaflets and the production of virus-like symptoms at the growing points (frenching). High applications of TIB may cause stunting of the plant, with lateral shoots showing a constriction at the point of emergence from the leaf axil; at low rates of treatment there may be elongation of the internodes and stem curvature. The compound would appear to be translocated to the growing points where it induces the development of abnormal leaves and the formation of an unusually large number of flower primordia in inflorescences initiated after treatment. There is general suppression of vegetative growth correlated with the increase in flowering activity.

De Waard and Roodenburg (19) also working with tomatoes, applied aqueous solutions of TIB to the growing points of seedlings. The treated plants remained small and produced terminal clusters of flower primordia without undergoing normal vegetative pre-flowering growth. Owen (9), however, found no effect on the flowering of tomatoes when TIB was applied to the soil, though certain modifications occurred in vegetative growth.

Since the effects of TIB on the growth of tomatoes were first reported (26) the action of this substance upon other plants has been studied. No outstanding flower responses were induced (2, 3, 4, 10, 16, 22). In agreement with these findings, experiments on hops carried out at Wye over the past three years, failed to produce any effect on flowering, though fasciation of leaflets was common and, in extreme cases, trumpet-like leaves were produced encircling the stem.

During the course of our investigations on growth-regulating activity in relation to chemical structure, we have prepared a wide range of aryloxyaliphatic acids. For assessing the activity of these compounds a number of tests have been employed, including the tomato leaf epinasty test. Certain substances, primarily applied for this test, were found to induce marked morphological changes in the subsequent growth of the plant. It is the purpose of this paper to describe these effects. One compound, α-(2-naphthoxy) phenylacetic acid (NPA), was found to be of particular interest in that it produced somewhat similar effects in the tomato to those induced by TIB. Comparative tests on the tomato, using these two compounds, are described.

MATERIALS AND METHODS.

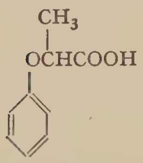
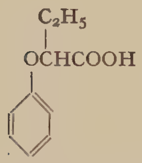
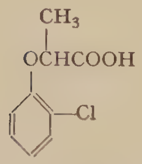
Synthesis of aryloxy acids.

α-(2-naphthoxy) phenylacetic acid (NPA), a compound not previously described in the literature, was prepared, as follows, by condensation of the sodium salt of β-naphthol with ethyl α-bromophenylacetate. Phenylacetic acid was brominated in the presence of red phosphorus by the method of Hell-Volhard-Zelinsky and the product refluxed with excess ethyl alcohol to give the ethyl α-bromophenylacetate, b.p. 138-142° C. at 11 mm. (yield 85 per cent. theoretical).

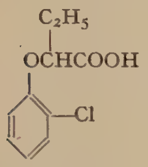
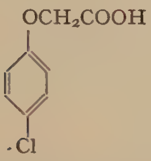
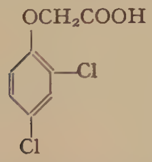
Metallic sodium (0·15 mole) was dissolved in 120 ml. ethyl alcohol and β-naphthol (0·15 mole) dissolved in the solution. The above bromo-ester (0·15 mole) was added and the solution heated under reflux for 2-3 hours. Excess 2 N sodium hydroxide was added and heating continued until hydrolysis was complete.

TABLE I.

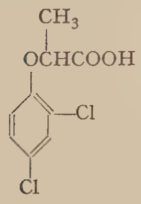
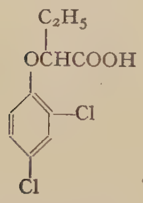
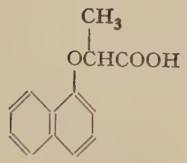
Morphogenic effects induced by treatment with lanolin solutions.

Compound.	Melting point °C.	Equivalent weight.*	Morphogenic effect.	References to previous work on tomatoes.
 α-phenoxypropionic acid.	114–114·5	165·9 (166·1)	2 per cent. caused epinasty within 24 hours. Stem bending, downward curvature of treated petiole and twisting of leaflets pronounced. Effects still marked after 6 days; after 13 days, treated leaf still epinastic though recovering. Inflorescence directly above treatment ceased to grow, flower buds turning yellow and abscissing. Compound appeared to be translocated to growing point since new leaves were small and dark green with elongated rachis. Flower buds on later inflorescences were profoundly modified with fusion of the sepals to form an elongated tube with a small opening at the distal end. Buds with sepals completely fused were common and these failed to open. Flower buds with sepals only partially fused set elongated and plum-shaped fruit somewhat smaller than normal.	27
 α-phenoxy-*n*-butyric acid.	80	179·5 (180·1)	2 per cent. induced epinasty within 24 hours. All the leaves above treated region showed severe epinasty and curling: some stem bending. Epinastic responses and formative effects on new leaf growth similar to those induced by α-phenoxypropionic acid. After 4 weeks, much callus formation was evident on treated part of stem, but there was no formation of root primordia.	27
 α-(2-chlorophenoxy)-propionic acid.	113·5–114·5	200·2 (200·6)	At 1 per cent. general petiole bending and curling of leaves above the treatment occurred within 24 hours. After 1 week, stem was swollen and developing white patches under the epidermis, while the growing point appeared stunted. Within 1 month, these white patches had given rise to numerous root primordia; these were especially evident in region of application. New growth appeared normal.	6, 27

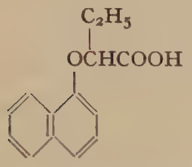
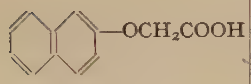
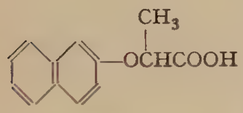
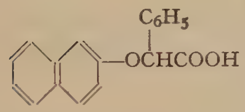
* Figures in parentheses indicate theoretical equivalents.

Compound.	Melting point °C.	Equivalent weight. *	Morphogenic effect.	References to previous work on tomatoes.
 α-(2-chlorophenoxy)-*n*-butyric acid.	81·5–82·5	213·7 (214·6)	1 per cent. caused petiole and stem bending and general epinasty over whole plant within 24 hours. After 1 week, many white patches developed on stem below point of application. After 1 month, these patches gave rise to many root primordia, with the resultant sloughing off of epidermis and cortex, the effect being more pronounced than with α-(2-chlorophenoxy)propionic acid. Plant very stunted, but no morphogenic effects on leaves.	6
 4-chlorophenoxyacetic acid.	157–158	184·9 (186·6)	At 1 per cent. induced curling and epinasty of treated and other leaves within 24 hours. After 6 days, severe frenching was evident at the apex; leaflets were dark green, reduced in area and thicker than normal. There was slight elongation of the rachis. White patches appeared on the stem in treated region and the plant appeared stunted. Inflorescences differentiated after treatment produced flower buds with fused sepals, some being modified to a narrow tube with small distal opening, others were completely closed. Plum-shaped fruits on these trusses were not uncommon; these resembled the modified fruit produced by α-phenoxypropionic acid. After 1 month, the leaves produced at the growing points were almost normal in appearance.	25, 27
 2 : 4-dichlorophenoxyacetic acid.	141	220·8 (221·1)	At 1 per cent. a marked epinasty was apparent in all leaves within 6 hours. Compound was apparently translocated rapidly causing curling of leaflets and much stem bending. Effect still marked after 10 days, by which time white patches had formed under stem epidermis which later split to expose the proliferating cortex. After 13 days epinastic leaves and petioles had partially recovered but growing point had become stunted and new leaves were slightly frenched. These same leaves were still curled and epinastic after 1 month. There was no root formation.	11, 13, 25, 27

* Figures in parentheses indicate theoretical equivalents.

Compound.	Melting point °C.	Equivalent weight. *	Morphogenic effect.	References to previous work on tomatoes.
 α-(2 : 4-dichloro-phenoxy)propionic acid.	116–118	235·6 (235·1)	At 1 per cent. initial responses as marked as those recorded for the acetic derivative, but after 3 days, though morphogenic effects on leaves and proliferation of the cortex were similar, the effects were less marked. Recovery then ensued and new growth after 1 month appeared normal. Browning and necrosis developed in the treated region. No root formation occurred.	13, 27
 α-(2 : 4-dichloro-phenoxy)-*n*-butyric acid.	80·5–81	248·6 (249·1)	At 1 per cent. epinastic responses shown within 24 hours. Growing point was hardly affected and new leaves showed only slight modifications though smaller, darker green and thicker. Responses generally similar to the two preceding compounds but much less marked. After 4 weeks, growing point almost normal, but bad necrosis had developed at point of application. There was no root initiation.	27
 α-(1-naphthoxy)-propionic acid.	150–151	217·6 (216·1)	At 2 per cent. gave no response after 24 hours, but within 48 hours epinasty was apparent. After 3 days this was more pronounced, and after 6 days there was frenching, darkening and curling of leaflets at the growing point. By the 8th day, effect of treatment was marked ; area of new formed leaflets reduced with proliferation of the midribs, vein clearing and thickening, and darkening of the lamina. As these leaves enlarged, there was considerable elongation of the rachis and petiole, while the proliferation of the midribs caused the formation of pitcher-like leaflets. The photosynthetic area of these leaves was small, each showed the marked development of epidermal hairs and the dark green colour associated with virus-affected plants. Internodes were much elongated and callus formed on the treated part of the stem and petiole. No root primordia were induced. Flowering was not affected but many of the later formed fruits were plum-shaped.	

* Figures in parentheses indicate theoretical equivalents.

Compound.	Melting point °C.	Equivalent weight. *	Morphogenic effect.	References to previous work on tomatoes.
 α-(1-naphthoxy)-*n*-butyric acid.	109·5–110·5	229·6 (230·1)	At 2 per cent. effects were similar to those shown by the corresponding propionic derivative, but less marked. There was greater production of callus and some necrosis where compound had been applied. No initiation of root primordia.	
 2-naphthoxyacetic acid.	155–156	203·6 (202·1)	At 1 per cent. gave an epinastic response of the treated petiole, and stem bending within 6 hours. Progressive leaf curling and bending continued for the next 2 days, after which recovery began. Slight darkening of new leaf tissue noticeable, but growing point not appreciably affected. After 13 days white patches appeared on the stem and petiole at point of treatment, developing into mass of root primordia within the next 3 weeks.	1, 24, 27
 α-(2-naphthoxy)-propionic acid.	106–107	215·3 (216·1)	At 1 per cent. responses induced were indistinguishable from those induced by the previous acetic derivative, though root primordia were more plentiful.	27
 α-(2-naphthoxy)-phenylacetic acid.	153	278·4 (278·1)	At 1 per cent. induced depression of treated petiole within 24 hours. After 4 days, much stem-bending and leaves at apex frenched. Later formed leaves showed fasciation of leaflets. Lateral shoots above points of application reduced to one or two small leaves having 3 to 5 tiny leaflets, and a terminal knob of flower primordia. Effects not unlike those recorded by other workers for plants treated with TIB.	

* Figures in parentheses indicate theoretical equivalents.

The alcohol was distilled off, the acid precipitated by the addition of hydrochloric acid and crystallized twice from petroleum, b.p. 80-100° C. α-(2-naphthoxy)-phenylacetic acid was thus obtained as white needles, m.p. 153° C. Equivalent weight, 278·4 ($C_{18}H_{14}O_3$ requires 278·1). Yield 55 per cent., calculated from the weight of bromo-ester used.

All other compounds used in these investigations were made similarly by condensing the sodium salt of the phenol with the ethyl ester of the appropriate α-bromo acid followed by alkaline hydrolysis, acidification and crystallization. Melting points and equivalents are given in Table I.

Preparation of materials for use.

Lanolin solutions of the free acids were made up at 1 or 2 per cent. by dissolving a weighed quantity of the compound in the melted lanolin.

Soil applications were made by watering the plants in 4-inch pots with 50 ml. of aqueous solutions of the sodium salt of the acid.

Sprays were usually applied as aqueous solutions of the sodium salt with the addition of 0·33 per cent. of a proprietary wetting agent of the secondary alcohol sulphate type. In some experiments, fine suspensions of the free acid in water were used. These were prepared by the addition, with stirring, of an alcoholic solution of the acid to water containing wetting agent to give a suspension containing 2 per cent. alcohol and 0·33 per cent. wetting agent.

Plants were given an overall spray to run-off, using a small hand atomizer.

The tomato leaf epinasty test.

This test described by Zimmerman and Hitchcock (23, 13, 14) involves the application of a lanolin solution of the growth substance to the adaxial surface of the petiole and to the side of the stem above the petiole of the fourth leaf from the apex of a 6 inch tomato plant.

RESULTS.

Compounds inducing morphogenic effects.

Some 36 aryloxyaliphatic acids were applied in lanolin to tomato plants as for the leaf epinasty test and observations were made on the growth and morphology of the plants during at least 6 weeks following treatment. Only those compounds listed in Table I caused morphogenic responses at the concentrations employed.

Compounds not inducing morphogenic effects.

When applied in 1 per cent. lanolin solution, as in the tomato leaf epinasty test, the following results were obtained :

Epinastic response but no subsequent morphogenic effects.	No epinastic or morphogenic responses.
2-chlorophenoxyacetic acid.	Phenoxyacetic acid.
α-(4-chlorophenoxy)propionic acid.	α-phenoxyphenylacetic acid.
1-naphthoxyacetic acid.	α-phenoxymalonic acid (disodium salt).
α-(2-naphthoxy)-*n*-butyric acid.	α-(2 : 4-dichlorophenoxy)phenylacetic acid.
α-(4-chlorophenoxy)-*n*-butyric acid.	α-(1-naphthoxy)phenylacetic acid.
	α-(2-naphthoxy)-*n*-valeric acid.
	α-(2-naphthoxy)malonic acid (disodium salt).
	β-phenoxypropionic acid.
	β-(2 : 4-dichlorophenoxy)propionic acid.
	β-(1-naphthoxy)propionic acid.
	β-(2-naphthoxy)propionic acid.
	Phenoxy*iso*butyric acid.
	2-chlorophenoxy*iso*butyric acid.
	4-chlorophenoxy*iso*butyric acid.
	2 : 4-dichlorophenoxy*iso*butyric acid.
	2-methyl-4-chlorophenoxy*iso*butyric acid.
	1-naphthoxy*iso*butyric acid.
	2-naphthoxy*iso*butyric acid.

FURTHER EXPERIMENTS.

α-(2-naphthoxy)phenylacetic acid (NPA) and 2 : 3 : 5-triiodobenzoic acid (TIB).

Seedling tomato plants, var. *Radio*, having cotyledons expanded and first two leaves appearing were treated as follows :

1. *Drop Method* (19).

0·05 ml. quantities of aqueous suspensions containing 20, 200 and 500 p.p.m. of NPA were applied to the growing points, by means of a micro-pipette.

2. *Spray application.*

Seedlings were sprayed with aqueous suspensions containing 10, 100 and 250 p.p.m. of NPA.

3. *Soil application.*

50 ml. quantities of aqueous solutions of the sodium salt of NPA were applied to seedling plants in 4 inch pots, to provide the equivalent of 2, 20 and 50 mg. of acid per pot.

Treated and control plants were grown in the glasshouse for 6 weeks and records taken at intervals. The results are given in Table II.

TABLE II.

Responses of tomato seedlings to treatment with NPA.

Treatment.	Responses after 13 days.	Responses after 24 days.	Responses after 6 weeks.	No. of flower buds on first truss.
Drop method 20 p.p.m.	Reduction in epicotyl elongation and leaf expansion. Leaves dark green and slightly wrinkled (frenching).	Plant almost normal except for slight frenching of first two leaves.	Plant apparently normal.	8
200 p.p.m.	Plant similar to control. Leaves expanding, epicotyl normal. Slight frenching.	Slight frenching of first two leaves. Fasciation of leaflets on third, fourth and fifth leaves. Sympodial growth.	Plant normal, apart from original growth.	11
500 p.p.m.	Marked elongation of epicotyl. Leaves curled and frenched, dark green and generally epinastic.	Frenching of first two leaves and marked fasciation of leaflets with midrib proliferation in later leaves. First inflorescence terminal with small shoot developing laterally.	Lateral shoot developing producing normal growth. Node-like swellings on flowering axis.	28

Treatment.	Responses after 13 days.	Responses after 24 days.	Responses after 6 weeks.	No. of flower buds on first truss.
Spray application 10 p.p.m.	Pronounced elongation of epicotyl, leaves epinastic but otherwise not malformed. No frenching.	Plant apparently normal.	Plant apparently normal.	10
100 p.p.m.	Marked elongation of of epicotyl, leaves epinastic and dark green with much frenching. Growing point affected.	Reduction in area and frenching of first two leaves. Fasciation and midrib proliferation in next five leaves. Flower truss terminal with small lateral shoot.	Lateral shoot growing normally. Other leaves showing marked fasciation. Node-like swellings along axis of terminal truss.	14
250 p.p.m.	Elongation of epicotyl, but little epinasty. Leaflets badly frenched and curled. Growing point affected.	First and second leaves frenched, rest much modified, showing leaflet fusion and midrib proliferation. Inflorescence growing terminally and vegetative apex suppressed to small lateral shoot.	Lateral shoot growing normally. Other leaves showing marked fasciation. Node-like swellings along axis of terminal truss.	17
Soil application 2 mgm.	Plant apparently normal.	Plant apparently normal.	Plant apparently normal.	13
20 mgm.	Growth much stunted compared with control.	Little further growth.	After 4 weeks growth recommenced. First two leaves much frenched. Next five showed typical fasciation of leaflets and midrib proliferation. No sign of inflorescence.	—
50 mgm.	Complete stunting, no growth following treatment.	No growth, plant only 1½ in. high.	No further growth. Plant, alive and green, still 1½ in. high.	—
Control plant.	Two leaves expanded.	Seven leaves expanded; lateral inflorescence developing.	Thirteen leaves expanded, inflorescences developing laterally.	7

Comparative investigations with NPA and TIB.

Lanolin Treatments. An experiment, using lanolin solutions of NPA and TIB, was carried out on 6-inch tomato plants, var. *Stonor's Exhibition.* A measured area of stem internode was treated with equal quantities of each acid as follows :

(*a*) 0·5 mg. applied unilaterally to 1 sq. cm.
(*b*) 1·0 mg. ,, ,, ,, 1 sq. cm.
(*c*) 2·0 mg. ,, ,, ,, 2 sq. cm.
(*d*) 3·0 mg. ,, ,, ,, 3 sq. cm.

After 4 days, each treatment produced a marked bending of the stem at the nodes, a slight depression of the petioles, and darkening of the young leaves at the growing point. After 12 days, all plants showed severe stem bending at the nodes and frenching of the apical leaves. At this stage, all plants, whether treated with NPA or TIB, had a similar appearance. The two higher applications of TIB later produced a marked stunting of growth, the internodes between later-formed leaves being considerably shorter than those of control or corresponding NPA treated plants, also frenching of the leaves was more persistent. Some of the leaves and lateral shoots on these TIB treated plants showed a typical constriction at the point of junction with the stem, an effect which was never observed on NPA plants. The internodes of all plants receiving NPA were equal to, or slightly more elongated than those of the controls, while the later-formed leaves showed much less frenching but more leaflet fusion and midrib proliferation than the TIB plants. The lower rates of application of both NPA and TIB gave similar responses. Leaves enlarging at the time of treatment remained small and frenched ; those formed later were larger but fasciation of leaflets and midrib proliferation was common.

After 5 weeks, all plants treated with NPA or TIB had developed a terminal inflorescence, and lateral shoots formed after treatment either terminated directly in a knob-like cluster of flower primordia (especially noticeable with the higher applications of TIB) or produced the terminal flower cluster after the formation of one or two much reduced leaves. With the two higher TIB applications the growing point lateral to the terminal inflorescence remained dormant. With the two lower applications of TIB and with all NPA treatments, however, these growing points developed and showed normal sympodial growth.

The terminal trusses generally produced a larger number of flower primordia on the TIB plants than on NPA or control plants.

Anatomical examination of treated plants. Both NPA and TIB induced the production of node-like swellings along the length of the flowering axis. Sections through these nodes showed a proliferation of the internal phloem, the latter appearing as scattered islands surrounded by small-celled parenchyma. Within, and surrounded by this wide ring of internal phloem and parenchyma, was a normal large-celled pith.

Dr. Gorter (5) has shown that tomato plants treated with TIB in the seedling stage may develop a flowering axis with two vascular rings, one inside the other. This phenomenon was not apparent in any of our TIB plants but was noticed in one plant treated with NPA. A terminal truss on this plant appeared to develop a sunken apex with a ring of small flower primordia round the edge. Sections of this flowering axis cut in sequence from the base to the apex showed, at first, a normal differentiation of the vascular system. Sections nearer the apex showed an occasional medullary bundle consisting of a central xylem vessel surrounded by phloem. These bundles became larger and more numerous nearer the apex, smaller bundles fusing to form a single larger one until the orientation was that of a ring of medullary bundles within the normal vascular system. Near the apex of the flowering axis, the internal ring of bundles completely fused to form an entire ring of external phloem, cambium, xylem and internal phloem, while sections just below the apex showed the initial development of yet a third vascular ring.

Spraying experiments. 6-inch tomato plants, var. *Monarch* were sprayed with aqueous solutions of the sodium salts of NPA and TIB at M/4,000, M/8,000 and M/20,000, two plants being used per treatment. Plants were grown on in a glass-house and observations were continued for some 6 weeks.

Three days after treatment, the typical frenching and stem bending induced by these compounds was apparent, the plants treated with the highest concentrations appearing most affected. As the plants developed, all the NPA treatments induced elongated internodes and later-formed leaves showed a high degree of fasciation at all concentrations. Terminal inflorescences were produced on all NPA plants, both on the main axis and on lateral shoots.

The highest concentration of TIB induced stunting of the plant and a retardation of vegetative growth, while new leaves showed frenching and constrictions became apparent where new leaves and lateral shoots joined the stem. These constrictions were not observed in NPA plants. Plants which had received the two lower concentrations of TIB were not stunted, though fasciated leaves, terminal inflorescences and node-like swellings on the flowering axis, similar to those shown by all NPA plants, were produced. A larger number of flower primordia was, however, differentiated on all the TIB plants.

Other workers have shown that TIB can suppress the activity of indole-3-acetic acid in the standard Avena test under certain conditions (3, 18) and that it can inhibit elongation in the oat cylinder test (15). Slight activity has been recorded in the Went pea test (15). It is of interest to mention here, that we have found NPA to be active in the Went pea test and to be strongly inhibiting in the Avena cylinder test when used at 28 p.p.m. (10^{-4} *M*).

DISCUSSION.

The results presented in Table I, show that the application of certain aryloxyaliphatic acids may produce decided morphogenic effects when applied to tomato plants. Certain of these effects have previously been reported by other workers (Table I). It is probable that a large number of compounds are capable of producing modifications in growth when applied at high concentrations and it is therefore important to note that the results given in this paper apply only to the treatments stated.

The results recorded do not enable any clear correlation to be drawn between chemical structure and morphogenic responses, although it is perhaps interesting that α-phenoxypropionic acid and α-phenoxy-*n*-butyric acid both induced frenched leaves with very elongated rachis. Again, the formation of leaflets modified to a pitcher-like shape, with a reduced photosynthetic area and proliferated midribs, was induced by α-(1-naphthoxy)propionic acid and α-(1-naphthoxy)-*n*-butyric acid. In general, however, the morphogenic responses obtained could not be related to ring or side-chain structure. Thus, for example, α-phenoxypropionic acid, α-(1-naphthoxy)propionic acid and 4-chlorophenoxyacetic acid all induced the formation of plum-shaped fruits; α-(2-chlorophenoxy)-propionic acid and 2-naphthoxyacetic acid induced root primordia in the treated region; α-(2-naphthoxy)phenylacetic acid and 4-chlorophenoxy-acetic acid induced the frenching of leaves, typical of virus-affected plants.

It is interesting that all *iso*butyric and β-propionic compounds tested were uniformly inactive. The inactivity of these *iso*butyric acids has been the subject of further work in these laboratories, involving the biological assessment of the compounds by several methods, including the Went pea, oat cylinder and standard Avena tests. These results will be discussed in a subsequent paper. Synerholm and Zimmerman (14) have produced evidence which suggests that β-(2 : 4-dichlorophenoxy)propionic acid may be converted to the parent phenol by a β-oxidation mechanism in the plant. Such an oxidative breakdown may account for the inactivity of this compound as a growth substance and may also explain the apparent inactivity of the other β-substituted propionic acids used in the present investigations.

Aryloxyaliphatic acids have found use in tomato culture to supplement the natural setting of the fruit, but it is clear that responses such as frenching of leaves, stunting of growth and fasciation of leaflets are features to be avoided in a commercial fruit-setting spray.

One of the compounds most widely employed for this purpose is 2-naphthoxyacetic acid. This substance, although capable of inducing morphogenic and epinastic effects (Table I) can produce an excellent set of good quality tomatoes when applied as a spray containing some 40 to 60 p.p.m. to the open flower truss, and used at this concentration, detrimental effects are not usually encountered in horticultural practice. This compound therefore possesses an adequate margin of safety in use (20). This does not apply, however, to α-(2-naphthoxy)propionic acid when used at a similar concentration on indoor plants (20). Although active in setting tomatoes, we have found that α-phenoxypropionic acid, α-(1-naphthoxy)propionic acid and α-(2-naphthoxy)phenylacetic acid may similarly cause some frenching and stunting of indoor plants at concentrations which would be necessary for fruit setting.

Although a number of aryloxyacetic acids with substituent groups in the side chain has been examined as growth-regulating substances, as far as we are aware, no such compounds containing phenyl groups in the side chain have been studied. We have synthesized and examined a number of these compounds, however, and have shown that α-(2-naphthoxy)phenylacetic acid (NPA), when applied to the tomato plant, can induce morphological responses which, in many respects, are similar to those produced by 2 : 3 : 5-triiodobenzoic acid (TIB). Thus, plants treated with NPA showed the frenching and fasciation of leaflets, formation of flower trusses which retained their terminal position during subsequent growth, and development of node-like swellings on the flowering axis, all of which are well known responses to TIB treatment. Although TIB has been shown to increase the number of flower primordia in the tomato (28), the increase obtained by NPA treatment (Table II) may not, however, be significant in view of the small number of plants used.

In general, TIB is quantitatively more active than NPA in the production of morphogenic responses in the tomato plant. Further, the effects of TIB treatment appear to be more persistent than those of NPA. Nevertheless, it is of interest that these somewhat unique morphogenic effects should be induced by two compounds which are structurally different. Thus, only α-(2-naphthoxy)phenylacetic acid

(NPA) possesses the aryloxy acid type of structure which fulfils the various requirements put forward by other workers (7, 8, 17) for active plant growth-regulating substances.

SUMMARY.

A range of compounds of the aryloxyaliphatic acid type has been synthesized and the capacity to induce morphogenic effects on the tomato plant has been studied. Plants were treated by the application of lanolin solutions to parts of a leaf petiole and stem, and growth responses recorded over some 6 weeks. At the concentrations used certain of these substances produced epinastic and morphogenic responses, some of which had previously been recorded by other workers. Other compounds produced epinasty only; the remainder were completely inactive.

α-(2-naphthoxy)phenylacetic acid (NPA) was found in these experiments to produce responses somewhat similar to those shown by tomato plants treated with 2 : 3 : 5-triiodobenzoic acid (TIB). These results were confirmed in a series of comparative experiments using different methods of application. Tomato plants treated with NPA and TIB both show frenching and fasciation of leaves, and the development of inflorescences in the terminal position. Quantitatively, TIB was the more active compound.

ACKNOWLEDGMENTS.

Thanks are due to Mr. C. H. Fawcett, B.Sc. and to Miss R. D. Walker, M.Sc., for assistance in the chemical investigations.

The work was made possible by the award to one of us (D. J. O.) of a College Fellowship provided by Messrs. May & Baker, Ltd.

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STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES. II. SYNTHETIC COMPOUNDS INDUCING MORPHOGENIC RESPONSES IN THE TOMATO PLANT. BY DAPHNE J. OSBORNE AND R. L. WAIN.

PLATE I.

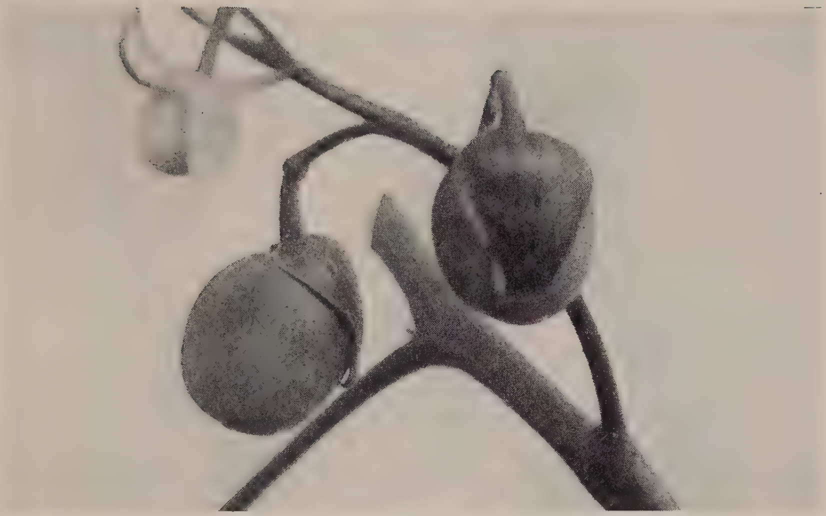

FIG. 1.

Plum-shaped fruits with fused sepals on plant treated with α-phenoxy-propionic acid.

[*face p. 72*

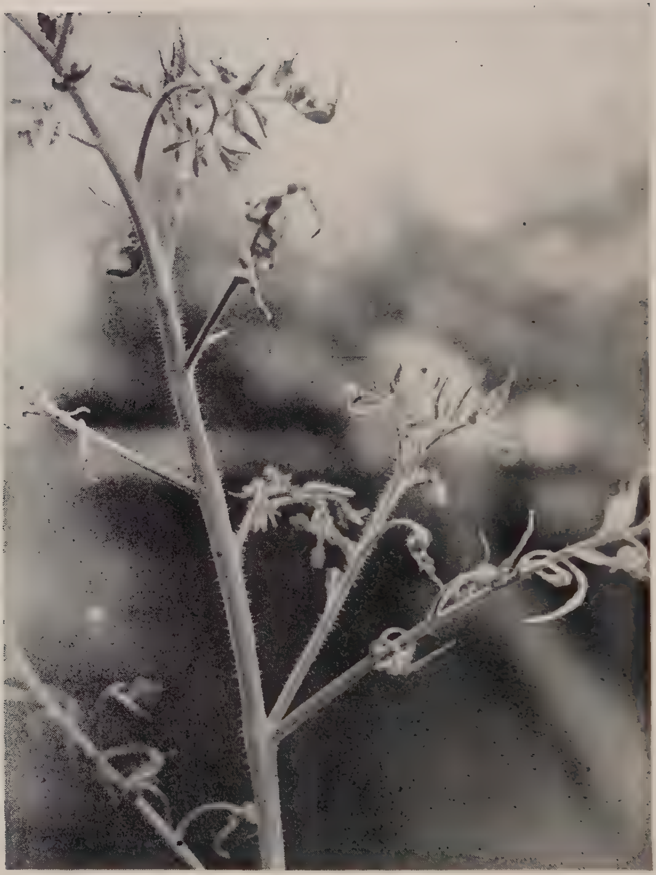

FIG. 2.
Leaf modifications on plant treated with α-(1-naphthoxy)-propionic acid.

PLATE III.

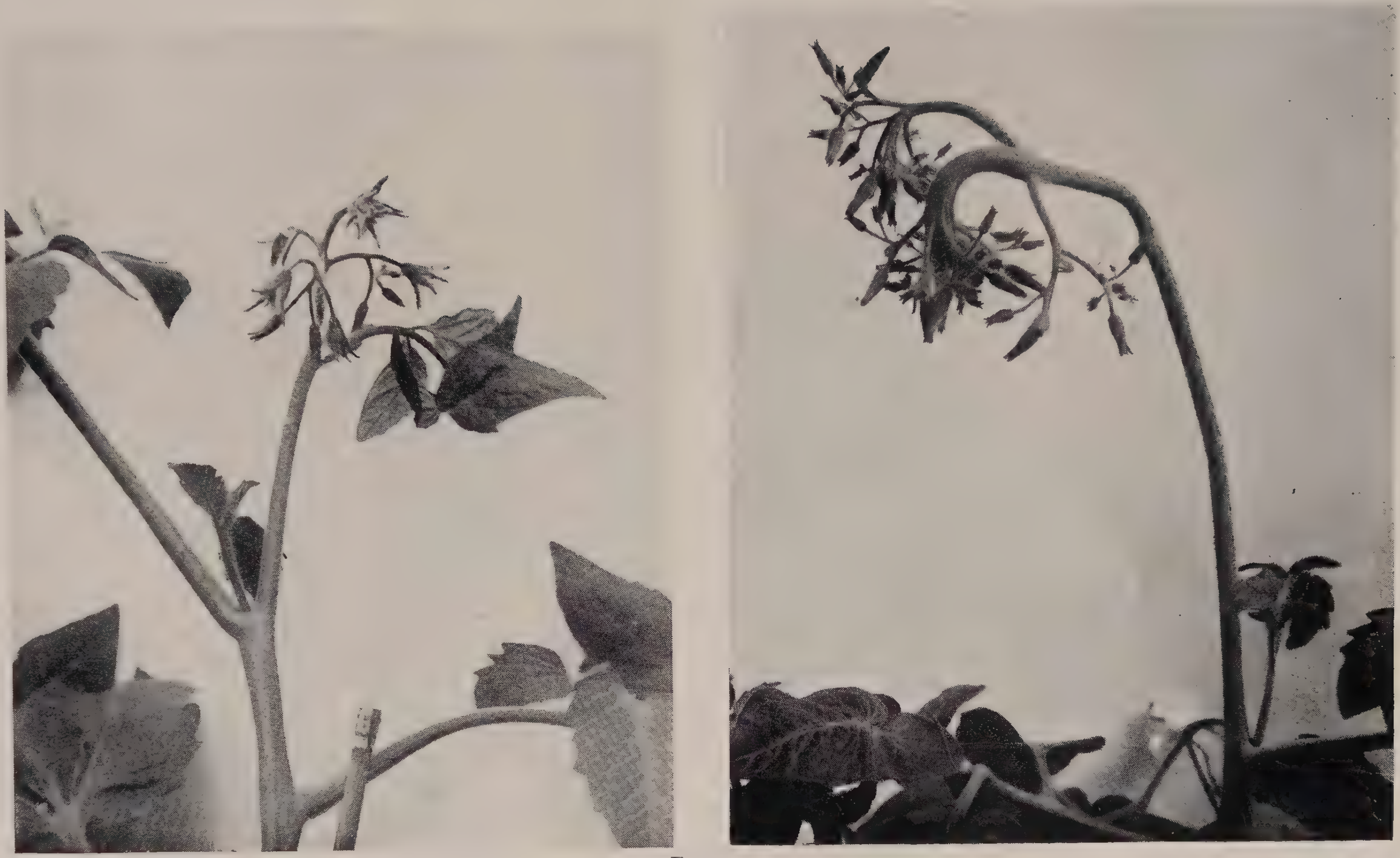

FIG. 3.
Terminal inflorescences induced by treatment with (*left*) NPA and (*right*) TIB.

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(*Received* 18/4/50.)

REVIEWS

JOURNAL OF EXPERIMENTAL BOTANY. An official organ of the Society for Experimental Biology. Editor, T. A. BENNET-CLARK. (London: Geoffrey Cumberlege, Oxford University Press. Vol. 1, No. 1. March, 1950. Subscription, 35s. per volume of three numbers.)

The appearance of a new botanical journal, devoted to the experimental aspects of the science, is a matter of the greatest interest to research workers and others interested in horticultural science, and it is a pleasure to welcome the first number of the *Journal of Experimental Botany*. It is sponsored by the Society for Experimental Biology, for the publication of papers from at home and abroad in plant physiology, biochemistry, biophysics, and experimental agronomy.

In the first number, F. G. Gregory *et al.* report investigations on transpiration, showing that relatively wide fluctuations in the water content of the leaf can occur without effect upon the rate of water loss. They suggest that the continued increase in the rate of transpiration, observed after water equilibrium has been attained, may be due to the effects of permeability changes superimposed upon those of stomatal regulation. Experiments demonstrating the extreme sensitivity of stomata to variations in the carbon dioxide content of the surrounding air are described by O. V. S. Heath, while W. T. Williams reports a further study upon stomatal movement, in relation to the moisture content of the atmosphere and the water supply of the epidermal cells. Investigations on the path of radioactive carbon in photosynthesis, carried out at the University of California, are described by A. A. Benson and M. Calvin.

Metabolism and growth studies are represented, A. Allsopp reporting a further study on the acid metabolism of *Aspergillus niger*, while R. Brown and J. F. Sutcliffe describe a technique for the study of extension growth of the root and its use to determine the effects of sugar and potassium on root extension.

It is a particularly healthy sign that interest and support sufficient to justify the launching of this Journal have been forthcoming. The Editorial Board is a very strong one and provides every indication that the high standard set in the first number will be maintained in the future.

F.R.T.

RECENT ADVANCES IN FRUIT JUICE PRODUCTION. By V. L. S. CHARLEY and others. (Technical Communication No. 21, Commonwealth Bureau of Horticulture and Plantation Crops, East Malling. 1950. Pp. 176. Price 15s. net.)

This is a continuation of the work by Dr. V. L. S. Charley and Dr. T. H. J. Harrison, published in June, 1939 and entitled *Fruit Juices and Related Products*. How timely the latter proved to be is shown by the success of the scheme for providing orange, blackcurrant and rose hip syrups during the last war to children and expectant mothers.

In the Foreword, the Right Hon. Viscount Bledisloe, P.C., G.C.M.G., K.B.E., writes: "The abnormally high standard of the health of the nation during this second period of world upheaval, anxiety and distress, during which Britain was one of the war fronts, is traceable (apart from the rigorously applied policy of food equalization) in no small measure to this timely emphasis on vitamins, and notably vitamin C, of which fruit juices and their related products are the best known and most effective vehicles to the human organism.

The truly remarkable advance made during the war period, and since, both in the consumption of these products and in the mode of their production and preservation, fully justifies the issue of this second Bulletin, ten years after that of the first."

In the last war nutrition not only had to be held at pre-war levels, but also had to be expanded. Many will remember the writings of Sir John Boyd Orr and others which emphasized that the consumption of fruit and vegetables, consequently fruit products, was far below what was necessary for the health of the nation.

This volume is concerned mainly in the great advances in the technique of manufacture by a young industry. They are clearly set out by Dr. Charley with photographs and diagrams and all new machines are fully described.

It was in 1937 at the Imperial Fruit Show at Birmingham that Dr. Charley, when still a research worker at Long Ashton, put up an exhibit showing the principles of apple juice production. Since those days, as he says in this book, few new principles have emerged, but there have been enormous strides in methods of production, not only by the improvement of plant, but also of materials used; great advances have been made in keeping the product and machines absolutely sterile. However, there is one notable new principle which is described and that is the development of the storage of fruit juice and syrups by freezing. In the U.S.A. the concentration of orange juice by freezing has been a tremendous factor in the disposal of the orange crop in the past few years.

The other contributors to the volume are C. Hunnikin of the A.P.V. Company Ltd., London, who writes on the latest methods of de-aeration and pasteurization, particularly in relation to the prevention of "off flavours" and the retention of ascorbic acid in the juice. A. Pollard, M.Sc., Ph.D., of Long Ashton Research Station, Bristol, contributes a chapter on the vitamin contents of juices. W. W. Reid, F.R.I.C., Head of the Research Department of the Laboratories of Messrs. H. W. Carter & Co., Ltd., writes a chapter on pectin-degrading enzymes and their implication in the fruit products industry. Messrs. F. E. Atkinson and C. C. Strachan also contribute a chapter on production of canned tomato, apple and grape juices in Canada and U.S.A.

Dr. Charley in his Introduction emphasizes that this book is a marshalling of all the latest information on production; in this he has succeeded, for information has been gathered from all parts of the world. He is, in fact, the acknowledged leader of this industry in England. Thanks are due to Messrs. H. W. Carter & Co., Ltd., of whom he is a Director, for allowing him to undertake this work and for making available his knowledge to all and sundry, which will do much to raise the level of world nutrition.

There are two aspects of fruit juices which are not touched upon; one is the economic and the other the nutritional. Both should be tackled in similar bulletins and it is to be hoped that before long Dr. Charley or some other worker will discuss these other two essential aspects of the fruit juice industry. Regarding the nutritional value of fruit juice, there are various hints and suggestions of quite startling attributes of apple juice from different parts of the world, and it would seem that an investigation by the Medical Research Council or Ministry of Health into this aspect would be well worthwhile, especially in these days when food is valued mainly by its calorific value and the first five discovered vitamins, whereas so little is known about the trace element requirements of nutrition and about the value of some of the more obscure vitamins. One can only hope that Dr. Charley himself will undertake this work or be successful in persuading someone else to do so.

There is no doubt that the fruit growing industry will benefit greatly from these recent advances in fruit juice production and fruit growers will welcome this publication. Another market is opening up for them but the fruit must be delivered to the juice factory at a satisfactory price, the product must be sold at a satisfactory price and the quality of the product must be safeguarded in order that the public may get not only a refreshing drink, but also a valuable food.

C.P.N.

EVERGREEN ORCHARDS. By W. H. Chandler. (London : Henry Kimpton. 1950. Pp. 452, with 66 illustrations. Price 42s. net.)

The success of the author of *Deciduous Orchards* in writing a companion volume to deal with the other half of the world's fruit trees shows an extraordinary fund of knowledge as well as an uncommon breadth of interest. Any book of this kind must necessarily be largely a compilation from published literature, and the 687 selected references given at the end are obviously only a fraction of the material that has been digested. But good compilation requires more than reading, and the field experience that has enabled Professor Chandler to select and arrange his material has had to be acquired in many lands and climates.

The book is intended primarily as a text for undergraduate teaching, " written with the hope that all good students of tree horticulture will want to see as much of the world as possible through their special field of study, and become acquainted with as much different tree behaviour as possible ". It is on that profession, therefore, that it is to be judged.

There are, in one sense, three books in one ; and each with little modification could stand by itself. The first, a quarter of the volume, is general and comprises three chapters on the tree and its fruit, evergreen orchard environment, and growing and training evergreen orchard trees. From the circumstance that many scientific horticulturists in the tropics have been trained in temperate regions, an appreciation of the comprehensiveness of horticultural principles is probably commoner among them than among those who know temperate conditions alone, and such readers may regard this general section as redundant ; but, for its purpose of widening the horizons of the student in a temperate country, it is useful.

The next 89 pages deal with the various forms of the genus *Citrus*, which, as the most important of the evergreen fruit species, might be expected to occupy a considerable part of the book. Within its compass the treatment is good and, again remembering its purpose, we need not attempt comparison with the bulkier works addressed more particularly to the citrus specialist.

Slightly more than half of the total space is devoted to a miscellany of tropical and subtropical species, the definition of orchard trees being liberally but justifiably stretched to allow inclusion of a chapter on tea, coffee and cacao, and another on coconut and African oil palm. It is this section that is likely to prove most interesting to the majority of readers, because it deals with a field where good general texts are few ; and though it is not offered as a specialist treatment of tropical horticulture it is better, even if judged as such, than any recent text we have seen. For undergraduate teaching it should prove valuable in tropical as well as temperate schools ; whilst for its primary purpose it is wholly admirable and stands alone.

It is a pity that this book, printed in America and in most respects very well produced, is marred by numerous misprints ; beginning with " dicotylidinous " on p. 12 and continuing with inexcusable frequency throughout to " *Fusarium oxysporium* " on p. 418. Some of the writing, also, seems to have been rather hurried, and careful editing could improve the future editions that are likely to be required.

E.E.C.

IODINE AND PLANT LIFE. (Chilean Iodine Educational Bureau, Stone House, Bishopsgate, London, E.C.2. 1950. Pp. 114. Refs. 794. 7½ by 10 in.)

This work consists of an annotated bibliography of the literature published in different parts of the world and its many languages, from 1813—soon after the discovery of iodine—to 1949 inclusive, together with a clear, though brief, review of the more important conclusions or deductions to be drawn from it.

A discussion of the varying, but on the whole large, amounts of iodine found in seaweeds is followed by one on the content found in different land plants. It may be noted that watercress has a relatively high content, viz. 45 μg. per 100 gm. and that *Feijoa sellowiana* heads fruits with the remarkable content of 894 μg. per 100 gm.

There is evidence to show that the iodine content of plants can be increased by iodine application in the field or in pots. But whereas the average iodine content of a heavy textured fertile loam or clay or highly acid soil rich in organic material is naturally very high, that of sandy soils rarely exceeds 1 part per million. This should be borne in mind when preparing nutrient media.

The effect of spraying various iodine compounds on crops and of application to seeds is considered with reference to certain plants, including vegetables and hops. Iodine is contained in many of the new herbicidal growth substances and in particular it intensifies the herbicidal qualities of phenoxyacetic acid and its derivatives. It would appear not to have been used in hydroponic solutions except by Matlin of Los Angeles.

In plant protection it has proved useful in minor ways such as in the disinfection of potato cutting knives, and the success of solutions containing free iodine for the control of certain plant diseases is noted. Little success, however, has attended the use of iodides or iodates in the field. It has been successfully used for impregnating fruit storage wraps and crêpe paper plugs in boxes of transported grapes.

There is an author- and short subject-index. The book should prove most useful for reference.

D.A.

SPACING EXPERIMENTS ON VEGETABLES

I. THE EFFECT OF THE THINNING DISTANCE ON EARLINESS IN GLOBE BEET AND CARROTS IN CHESHIRE, 1948

By L. G. G. WARNE
Botany Department, Victoria University of Manchester

It is generally recognized for a great variety of crop plants that close spacing reduces the size of the individual plants but no precise information is available as to the stage of development at which inter-plant competition becomes important in reducing plant size. This is a point of major concern in the production of early root vegetables, where the aim is to obtain the maximum number of plants of a suitable size in the minimum time. Although crops for the production of early carrots, beet and turnips are generally sown more thickly or are thinned less severely (Hoare, 1945, Johnstone, 1948) than main crops, the possibility exists that this close stand of plants may so reduce the individual plant size that the date by which a reasonable number of plants may be pulled is actually postponed. To test this point carrots and globe beet have been thinned to four distances and harvested successionally.

Experimental procedure and results.

The experiments were carried out at the University of Manchester, Jodrell Bank Experimental Grounds, Lower Withington, Cheshire, on a heavy loam of high fertility. The site was winter-ploughed to a depth of 8 in., and limed in February, 1948, at the rate of 1¼ tons of hydrated lime per acre. No manures were applied in the year of the experiment. For both carrots and beet each plot consisted of a single row 12 ft. long. Four thinning distances were utilized and there were three harvests. The twelve treatments were arranged in six blocks, giving a total of 72 plots for each experiment.

Carrots.

Seed of the variety Early Wonder (Caldwell) was sown with a Planet Junior drill on 10th April in rows 18 in. apart. Germination was good, and the plants were thinned on 19th May. Good quality labour was available for the thinning and the men were provided with canes marked at appropriate distances and the four thinning distances employed were 1 in., 2 in., 4 in. and 6 in.

The three harvests were made on 23rd June (when few of the plants had reached an acceptable size), 7th July and 27th July. At each harvest, data were collected and were analysed separately. The results are assembled in Table 1. Two 6 in. plots were lost in the last harvest, and data for all the 6 in. plots (which are of minor interest only) for the third harvest are omitted from the Table. The roots were graded into (1) 1⅛ in. diameter or over, representing a size acceptable in mid-season; (2) ⅝ in. diameter or over, representing the minimum size of root acceptable early

TABLE I.

Yield and other data for carrots.

Size of plot, 12 ft. of row (= 18 sq. ft.).

Thinning Distance.	*1st Harvest (June 23rd).*				*2nd Harvest (July 7th).*				*3rd Harvest (July 27th).*		
	1″	2″	4″	6″	1″	2″	4″	6″	1″	2″	4″
Number of plants per ft. of row ..	10·11	6·14	3·76	2·47	10·45	6·87	4·05	2·59	11·16	6·9	4·38
Number of plants as % of theory ..	84	102	117	123	88	114	135	129	93	115	146
Total weight of roots, oz. per plot ..	13·9	10·4	4·9	3·3	55	41	24	10	141	118	83
Total weight of tops, oz. per plot ..	19·5	13·2	7·0	4·9	65	46	28	23	93	71	55
Weight of tops + roots, oz. per plot ..	33·4	23·6	11·9	8·2	120	87	52	42	234	189	138
Ratio $\frac{\text{Roots}}{\text{Tops}}$	0·71	0·79	0·70	0·67	0·85	0·87	0·86	0·85	1·52	1·67	1·51
Mean root weight, oz.	0·099	0·125	0·094	0·095	0·38	0·42	0·44	0·54	0·91	1·23	1·30
Mean top weight, oz.	0·138	0·157	0·133	0·144	0·44	0·49	0·50	0·63	0·60	0·74	0·84
Number of roots per plot over ⅝″ diam.	10·2	9·9	5·5	3·5	72	55	35	26*	144	90	57
Number over ⅝″ diam. as % total ..	7·2	11·6	10·5	10·0	49	57	61	73	93	93	93
Number of roots per plot over 1⅛″ diam.	—	—	—	—	—	—	—	—	49	51	35†
Number over 1⅛″ diam. as % of total ..	—	—	—	—	—	—	—	—	31	53	57

* Significant difference = 16.

† Significant difference = 11.

in the season, and (3) those of less than $\frac{5}{8}$ in. diameter, considered too small to be of value.

Reference to Table 1 shows that the number of plants present in the plots corresponds closely with the number expected. On the first harvest date the yields of tops plus roots and of tops and roots separately are almost proportionate to the number of plants present and at this stage the size of the individual plants appears to be unaffected by the thinning distance employed. The number of roots attaining a diameter of $\frac{5}{8}$ in. increases slightly as the number of plants present increases, but the number is too small to be of interest.

By the second harvest the plants show the effect of competition, and mean root weight and mean top weight is least at the closest spacing, although the total yields of both roots and tops are greatest at the highest plant densities. Although both mean root weight and the proportion of roots that exceed $\frac{5}{8}$ in. diameter is least, the number of roots over $\frac{5}{8}$ in. diameter is greatest at the closest spacings, and falls regularly as the thinning distance is increased. By the third harvest virtually all the roots have attained a diameter of $\frac{5}{8}$ in., and so the number falling in this grade is a direct reflection of the total number of plants present, but whilst the proportion attaining a diameter of $1\frac{1}{8}$ in. is greatest when the thinning distance is greatest, the number attaining this size falls significantly when the thinning distance exceeds 2 in.

The data are limited, but they support the custom of sowing thickly for the production of early carrots. Although by the time any great proportion of the crop reaches a marketable size, inter-plant competition reduces the mean plant weight, the number of roots reaching an acceptable size increases as the plant density increases the highest number being obtained with the thickest stand of plants employed in the experiment (eleven per foot of row). In practice, when, as is customary, the crop is selectively pulled several times, the closely spaced plots will show up even more advantageously than they have in these experiments, where it was necessary to harvest all the plants on a plot simultaneously.

Beet.

Seed of the variety Crimson Globe (Caldwell) was sown by hand on 10th April, 1948, in drills 18 in. apart. Germination was poor. The plants were thinned in early June, the nominal thinning distances employed being 2 in., 3 in., 4 in. and 6 in., and the three harvest dates were 24th June, 8th July and 28th July. The data collected at harvest are assembled in Table 2. The diameter chosen as representing the minimum acceptable size of root was $1\frac{1}{2}$ in. whilst it is probable that no advantage accrues from allowing the diameter to exceed $2\frac{1}{2}$ in. In all plots, but especially on the closer spaced ones, the number of plants present falls far short of the expected number, so that the range of plant densities obtained is small.

On the first harvest date no roots had reached a diameter of $1\frac{1}{2}$ in. and, at this date, increasing the thinning distance was without effect on the mean root weight or mean top weight so that the total yield per plot was proportionate to the number of plants present.

At the second harvest, mean root weight was reduced at the closer spacings and the ratio of $\frac{\textit{root weight}}{\textit{top weight}}$ increased as the thinning distance increased. The number of plants giving roots of a diameter of $1\frac{1}{2}$ in. or over was not significantly affected by the spacing.

Table II.

Yield and other data for Globe Beet.

Size of plot, 12 ft. of row.

Thinning Distance.	*1st Harvest (June 24th).*				Significant diff.	*2nd Harvest (July 8th).*				Significant diff.	*3rd Harvest (July 28th).*				Significant diff.
	2″	3″	4″	6″		2″	3″	4″	6″		2″	3″	4″	6″	
Number of plants per ft. of row ..	2·40	2·28	1·95	1·56		2·83	2·20	2·06	1·65		2·70	2·56	2·16	1·80	
Number of plants as % of theory	40	57	65	78		48	57	69	83		45	64	72	90	
Total weight of roots, oz. per plot	6·1	5·8	4·6	3·9	1·4	45·2	37·3	38·9	35·2	8	135	127	118	131	24
Total weight of tops, oz. per plot	22·7	25·4	17·9	16·0	1·8	79·6	62·7	64·3	54·6	13	112	105	90	89	15
Weight of tops + roots, oz. per plot	28·8	31·2	22·5	19·9		125	100	103	90		247	232	208	220	
Ratio $\frac{\text{roots}}{\text{tops}}$	0·27	0·23	0·25	0·24		0·57	0·60	0·61	0·64		1·21	1·21	1·31	1·47	
Mean root weight, oz.	0·18	0·19	0·17	0·18		1·12	1·24	1·39	1·54		3·57	3·63	4·05	5·35	
Mean top weight, oz.	0·67	0·70	0·65	0·74		2·03	2·10	2·25	2·39		2·97	3·01	3·15	3·60	
Number of roots per plot over ⅞″ diameter	7·0	7·8	4·9	5·6		—	—	—	—		—	—	—	—	
Number over ⅞″ as % of total ..	18	25	15	26		—	—	—	—		—	—	—	—	
Number of roots per plot over 1½″ diameter	—	—	—	—		15·1	12·6	12·5	12·2	4·0	30·8	28·7	24·6	24·1	6·1
Number over 1½″ as % of total ..	—	—	—	—		38	41	43	52		81	80	81	96	
Number of roots per plot over 2½″ diameter	—	—	—	—		0·7	1·8	2·2	6·3		21·8	19·6	19·6	19·7	5·2
Number over 2½″ as % of total ..	—	—	—	—		1·8	5·9	7·8	10·9		58	55	65	78	

By the third harvest the effects of competition in restricting plant growth and in altering the ratio of $\frac{\textit{root weight}}{\textit{top weight}}$ are still more evident but, even so, the number of roots attaining a diameter of 1½ in. is significantly greater on the nominal 2 in. than on the 4 in. or 6 in. plots. Thus, close spacing, as with the carrots, although it restricts growth and reduces the mean root weight, increases the number (although it decreases the proportion) of roots which attain an acceptable size ; this beneficial result will be accentuated if the crop is pulled selectively several times instead of being harvested simultaneously.

Although in this work we have been concerned with the number of plants attaining a certain size, the data relating to yields expressed as weight are not without interest. At the first harvest for both beet and carrots, the yield of roots per plot is almost directly proportional to the number of plants present but, as the season progresses, the dependence of yield on the number of plants present becomes less marked, and the mean root weight becomes more closely related to the space available per plant.

SUMMARY.

With globe beet and carrots, close spacing, by the time any reasonable proportion of the crop reaches a usable size, reduced the mean root weight. Nevertheless the largest number of roots of acceptable size was obtained with the closest thinning distances employed, viz. 1 in. for carrots and 2 in. for beet.

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(*Received* 22/2/1950.)

SPACING EXPERIMENTS ON VEGETABLES

II. THE EFFECT OF THE THINNING DISTANCE ON THE YIELDS OF GLOBE BEET, LONG BEET, CARROTS AND PARSNIPS GROWN AT A STANDARD INTER-ROW DISTANCE IN CHESHIRE, 1948

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PRECISE data on the relation between plant density and yield seem to be wholly lacking for our common root vegetables. With these crops the problem is one of great complexity. Whereas with many farm crops it matters little if a large number of small plants make up the total yield, with most root vegetables extremely large or small roots are disposed of only with difficulty, or in times of acute shortage. Generally, therefore, the aim is the maximum number and weight of intermediate sized plants. Weight alone is not always an adequate measure of yield. Some roots are sold bunched, when the number over a certain minimum size, rather than their weight, is the criterion of a satisfactory crop.

That an investigation of the effect of spacing on yield is long overdue is suggested by the diversity of thinning distances recommended in the literature. Commercial crops of carrots are usually not thinned (Hoare, 1945) but leaflets addressed to small growers suggest thinning distances of 4 in. or 6 in. (Ministry of Agriculture, 1939). Thinning distances suggested for parsnips are generally 6 in., 8 in. or 9 in. (Ministry of Agriculture, 1939, 1947), for early (globe) beet, from 2 in. or 3 in. up to 4 in. or 5 in. (Ministry of Agriculture, 1947), and for main crop globe and for long beet 6 in. or 9 in. (Ministry of Agriculture, 1939, and undated). It seems unlikely that the yield remains unaffected by such large variations in thinning distances as these.

The relation between plant density and yield may be investigated by two methods but the sampling technique employed by Engledow and his co-workers (1926, 1928, 1928*a*) and others (Forster and Vasey, 1931) was in this work rejected in favour of the alternative method of sowing thickly enough to give a regular stand of seedlings, and then thinning to predetermined distances. This procedure has been followed in the experiments on beets, carrots and parsnips which are described in the present paper.

EXPERIMENTAL PLOTS.

Location. All the experiments were carried out at the University of Manchester, Jodrell Bank Experimental Grounds, Lower Withington, Cheshire, on a heavy loam that had been generously manured for several years and was in a high state of fertility. No fertilizers were applied in the year of the experiments but the mean total yields of roots obtained were at the rates of approximately 17 tons per acre for parsnips, 14 tons and 13 tons for two varieties of long beet, 13 tons for globe beet, and 10 tons

TABLE I.

Yield and other data for Globe Beet and Parsnips.

Thinning distance.	No. of plants per foot of row.	No. of plants as % theory.	Large roots as % total.	Wt. of large roots.	Total wt. of roots.	Total wt. of tops.	Wt. of tops and roots.	Mean wt. of large roots.	Mean wt. of all roots.	Mean wt. of tops.	Ratio root/top.	No. of large plants per ft. of row.	Percentage of bolters.
				lb. per plot.				oz.					
Crimson Globe Beet.													
1 in.	5·83	49	74	24·4	26·0	14·4	40·4	3·76	2·97	1·64	1·81	4·31	1·14
2 in.	4·25	71	86	25·9	26·5	12·8	39·2	4·70	4·16	2·04	2·04	3·66	1·37
3 in.	3·33	83	86	22·3	22·9	10·6	33·4	5·17	4·57	2·12	2·15	2·86	1·50
4 in.	2·67	89	89	21·2	21·5	9·1	30·6	5·95	5·37	2·28	2·36	2·38	1·56
6 in.	2·17	109	90	19·7	19·9	8·0	27·9	6·70	6·13	2·46	2·49	1·95	3·46
Sig. Diff. (P=0·05)				**3·0**	**3·0**	**1·7**	**5·1**						
Parsnip, var. Tender and True.													
2 in.	4·41	74	59	20·8	26·8	15·8	42·6	7·09	5·39	3·19	1·69	2·60	—
3 in.	3·57	89	69	22·1	25·9	14·8	40·7	7·87	6·55	3·69	1·75	2·46	—
4 in.	2·88	96	80	23·1	24·8	14·6	39·4	9·00	7·73	4·49	1·71	2·30	—
6 in.	2·03	102	87	20·8	21·6	13·5	35·1	10·47	9·35	5·91	1·60	1·76	—
8 in.	1·71	114	84	18·6	19·4	12·9	32·3	11·41	10·00	6·67	1·51	1·43	—
Sig. Diff. (P=0·05)				**2·9**	**2·1**	**1·7**	**2·9**						

and 6 tons for two varieties of carrot. Corresponding values for yields of " large " (marketable) roots were 15 tons 3 cwt. for parsnips, 12 tons 2 cwt. and 11 tons 9 cwt. for the two varieties of long beet, 12 tons 5 cwt. for globe beet and 10 tons and 6 tons for the two varieties of carrot.

These yields may be judged satisfactory (there are special reasons for the low yields of carrots), especially as they include plots on which the spacings employed were far from the optimum and as no manures were applied in the year of the experiments.

Weather. The year of these experiments was characterized by a dry and forward spring. From mid-June onwards, apart from a week of hot weather at the end of July, cool, dull wet weather predominated until harvest. All the experimental crops grew satisfactorily.

General cultural methods. The site was winter ploughed to a depth of 8 in. and limed in February at the rate of 1¼ tons per acre. Spring cultivations were adequate to produce a satisfactory tilth for sowing. Sowing dates approximated to those usual in the district, and a standard inter-row distance of 18 in. was used. The plots were kept clean by hand cultivation. Good quality labour was available for thinning and for this operation the men were provided with canes marked at suitable intervals. In spite of these precautions, the number of plants present after thinning often fell far short of the theoretical number, especially at the close spacings.

Plot size. The plots employed were small. After discarding buffer rows and ends of rows, each plot consisted of a single row the length of which, whilst uniform for each experiment, varied from 12 to 24 feet. The plots were arranged in five or six randomized blocks.

EXPERIMENTAL PROCEDURE AND RESULTS.

Globe Beet.

Seed of the variety Crimson Globe (Caldwell), was sown by hand on 26th April, 1948. Germination was good, and a regular stand of seedlings was obtained. A small amount of Pygmy Mangel Beetle (*Atomaria linearis* Steph.) damage to the seedlings occurred, but the plants grew away from the damage after thinning in early June. The thinning distances employed were 1 in., 2 in., 3 in., 4 in. and 6 in., and counts made immediately after thinning showed that the plants present, as per cent. of the "theoretical" number, were 47 per cent., 66 per cent., 81 per cent., 83 per cent. and 99 per cent., respectively. Subsequently, a few plants bolted but the number was too small to interfere with the experiment.

The crop was harvested on 29th August, 1948, and the data obtained, at harvest, together with some statistics calculated from them, are assembled in Table I. The roots were divided into two grades—over and under 1⅞ in. diameter* (corresponding approximately to a weight of 2 oz.) and so, in the Table, "large" roots indicate roots of diameter over 1⅞ in.

Several points in the Table require comment. The number of plants present in the plot falls considerably short of the theoretical number, especially at the closer spacings, so that at the nominal 1 in. thinning distance only 5·8 plants are present per foot of row but as the thinning distance becomes greater, the discrepancies become less. All the plots, however, possessed at harvest more plants than the June count indicated. This increase in plant density is due in part to a small amount of delayed germination, and probably also to the omission from the June counts of a few plants that had been defoliated by Mangel beetle and to the counting as a single plant of two plants left in error at the same station. Together, these causes offer an explanation of the fact that, even at the wide spacings, approximately 10 per cent. of the plants failed to reach the large grade. The incidence of bolting was slight, but the percentage of plants bolting increases as the space per plant increases.

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* Compared with a 2" minimum diameter subsequently recommended by Ministry of Agriculture and Fisheries (Marketing Leaflet No. 102, 1948).

Total yields of tops and roots together and of tops alone decrease regularly as the number of plants per plot decreases, and although differences between yields for consecutive thinning distances often fail to reach the level of significance, the final picture is of a regular and significant decrease in yield as the thinning distance increases, the increases in the weights both of top and roots of individual plants being insufficient to compensate for the fewer plants present. The total yield of roots and of roots over $1\frac{7}{8}$ in. diameter likewise increases as the number of plants per plot increases up to the 2 in. nominal thinning distance, but beyond this (at 1 in.) there is a slight but not significant decrease. The effects of closer spacing on yield of top and root, whilst generally similar, are not identical ; the yield of tops is relatively more influenced by the spacing than is the yield of roots, so that as the spacing decreases, the ratio of $\frac{Roots}{Tops}$ decreases also. These effects might be explained if the plants which are closely spaced are not only smaller, but are physiologically younger than those at the wider spacings. The ratio of root to tops increases as the season progresses, and within limits this is true of the incidence of bolting. Hence the low value of the root to top ratio, and the low percentage of bolters found in the 1 in. plot, agree with expectations if these plants are physiologically younger than their wider-spaced counterparts.

Practically, the results are not easy to assess, since the minimum size of large roots is arbitrarily chosen as $1\frac{7}{8}$ in. diameter. A lowering of the grading limit would clearly be to the advantage of the close-spaced plots while a raising of the limit would affect them adversely. The mean weight of the roots over $1\frac{7}{8}$ in. diameter decreases regularly, as the spacing decreases from 6 in. to 1 in., and it is probably fair to assume that at the close spacings a bigger proportion of the roots would have to be discarded if the grading level were raised.

Conditions tending to increase the yield will presumably operate by increasing the mean root size, and by increasing the number of roots that attain the grading limit. But there is much scope for the latter increase only on the 1 in. plot. Higher yields therefore are likely to cause the 1 in. and possibly the 2 in. plots to appear in a more favourable light relative to the other plots. With lower yields the closely-spaced plots will be those most adversely affected. In this experiment, however, no advantage has accrued from increasing the thinning distance over a nominal 2 in. (giving 4·25 plants per foot of row). Indeed, at greater distances not only is the yield reduced, but some of the roots might be accounted undesirably large. Further, it will be noted that for each thinning distance employed, the number of large roots exceeds the total number of plants for the next wider spacing.

We find, therefore, no evidence justifying thinning distances of 5, 6 or even 9 in., even if at these distances the " theoretical " number of plants is obtained.

TABLE II.

Yield and other data for Long Beet.

Thinning distance.	No. of plants per foot of row.	No. of plants as % theory.	Large roots as % total.	Wt. of large roots.	Total wt. of roots.	Total wt. of tops.	Total wt. of tops and roots	Mean wt. of large roots.	Mean wt. of all roots.	Mean wt. of tops.	Mean wt. roots and tops.	Ratio root/top.	No. of large plants per ft. of row.
				lb. per plot.				oz.					
Var. Exhibition Black													
1 in.	6·33	53	49	11·2	14·9	5·5	20·4	4·76	3·15	1·16	4·30	2·72	3·10
2 in.	4·25	71	67	11·9	13·6	4·4	18·0	5·57	4·27	1·37	5·65	3·12	2·85
3 in.	3·50	88	69	11·6	12·7	3·8	16·5	6·42	4·85	1·44	6·29	3·37	2·42
4 in.	3·00	100	75	10·8	11·6	3·3	14·9	6·35	5·14	1·47	6·61	3·50	3·25
6 in.	2·42	121	86	11·1	11·4	3·1	14·4	7·09	6·28	1·69	7·97	3·71	2·08
Sig. Diff. (P = 0·05)				2·1	2·1	0·8	3·0						
Var. Cheltenham Green Top													
1 in.	6·58	55	45	10·6	13·8	6·7	20·5	4·67	2·78	1·35	4·14	2·06	2·96
2 in.	5·17	86	61	11·6	13·6	6·5	20·0	4·92	3·49	1·66	5·16	2·10	3·15
3 in.	3·67	92	72	10·9	11·6	5·4	17·1	5·31	4·31	1·97	6·28	2·19	2·64
4 in.	2·92	97	77	10·7	11·3	4·8	16·1	6·41	4·88	2·22	7·10	2·20	2·25
6 in.	2·25	112	80	9·4	9·8	4·2	14·1	6·96	5·77	2·48	8·30	2·33	1·80
Sig Diff. (P = 0·05)				2·3	1·6	1·1	1·8						

Long Beet.

Seed of the two varieties, Cheltenham Green Top (Caldwell) and Exhibition Black (Clibrans), was sown by hand on 26th April, 1948. Germination of both varieties was good, and a regular stand of seedlings was obtained. Counts of the plants immediately after thinning gave at the 1 in., 2 in., 3 in., 4 in. and 6 in. spacings 51, 78, 80, 93 and 104 per cent. of the theoretical number for Cheltenham Green Top, and 47, 67, 83, 94 and 108 per cent. for Exhibition Black. No bolting occurred, the plants grew satisfactorily, and they were harvested on 28th and 30th September, 1948. The data gathered are assembled in Table II. " Large " roots here are those of a diameter exceeding $1\frac{5}{8}$ in. (Approximate weight = 3 oz.). As with Globe Beet, the number of plants present, for the smaller thinning distances, falls considerably short of the expected number. The number of plants counted at harvest again slightly exceeds the June count.

Total yields of tops and roots, of tops alone and of roots alone all fall regularly as the density of the plant population decreases. As with Globe Beet the yield of tops is more sensitive than the yield of roots to the alteration in the thinning distance, so that the ratio of $\frac{Roots}{Tops}$ decreases as the thinning distance decreases. The percentage of plants in the large category increases regularly from the 1 in. to the 6 in. spacing, and might increase further with still wider spacings, although there is again a small residue of late germinators etc. amounting to 0·2 to 0·4 of a plant per foot of row, which would probably never attain the " large " size.

The plots were not laid out as a variety trial, and the significance of observed varietal differences cannot be assessed.

Practically, the chief interest is in the yield of large roots and this is, within the limits of the thinning distances employed, not significantly affected by the plant density with Exhibition Black ; with Cheltenham Green Top it falls only when the thinning distance exceeds 4 in., the yield at 6 in. being significantly less than that at 2 in. but not differing significantly from those at 1 in., 3 in and 4 in. As with Globe Beet, higher yields or a lower grading limit would appear to benefit the closely-spaced plots and vice versa.

The overall yields obtained (at the rates of 11·45 and 12·1 tons per acre) may be looked on as representing a normal crop, but the data obtained do not suggest the necessity of employing such wide spacing as the 6 in. or even 9 in. often recommended. Under these conditions, and with the chosen grading limits, spacings closer than the closest of those generally recommended, whilst not always beneficial, do not react adversely on the yields.

Parsnips.

Seed of the variety Tender and True (Caldwell), was sown by hand on 6th March, 1948. Germination was unusually good, and a regular stand of plants was obtained. The plants were thinned on 27th April, 1948, the thinning distances employed being 2 in., 3 in., 4 in., 6 in. and 8 in. Cultural details were as for the beet. Counts of the plants present in the plots in June gave respectively, 73, 89, 93, 102 and 112 per cent. of the expected number, and this agrees almost exactly with the counts of the plants harvested. The crop was harvested on 1st October, 1948, at which date the tops of the plants were dying back rapidly. Data collected at harvest and values calculated from these data are assembled in Table I. Large roots are those exceeding 2 in. diameter* (approximate weight = 5 oz.).

The general trends shown are very similar to those for the long beet. The percentage of plants falling into the large grade is decreased at the closest spacings, but total yields of tops + roots, of tops alone, and of roots alone decrease as the number of plants present decreases, and no regular change in the ratio $\frac{Roots}{Tops}$ occurs. Individual plants are largest at the greatest spacings, but this increased growth does not fully compensate for the smaller number of plants present. The yield of large roots is unaffected by the spacing until the 8 in. thinning distance is reached, when the yield is significantly reduced. Nothing, therefore, appears to be gained by increasing the thinning distance beyond 6 in., whilst reducing it below this level, although never recommended, does not appear to be disadvantageous, unless the grading limit is increased above 2 in., or unless plant growth is expected to be poor.

Carrots.

Seed of the varieties Early Wonder (Caldwell) and Altrincham (Caldwell), was sown with a Planet Junior drill on 9th April, 1948. Early Wonder germinated well, and Altrincham badly. The plants were thinned in early June, the nominal thinning distances being 1 in., 2 in, 3 in., 4 in. and 6 in.

Variety Early Wonder. The roots of this variety began to split badly in August, and the plots were, therefore, harvested on 23rd August, 1948. The plants were harvested in wet weather, and so no weights of the tops were obtained. By the harvest date some bolting—the amount of which seemed to be increasing—had occurred. Bolters were counted and removed, and are not included in the weights (see Table III). " Large " roots are those of a diameter exceeding 1⅛ in. (approximate weight = 1·5 oz.).

Spacing appears to affect the incidence of bolting, the percentage of bolters increasing as the thinning distances increase. Virtually, all the non-bolters attained the " large " size, but the percentage of these " large " roots, which were split, increased with wider thinning. The relation between the occurrence of splitting and the spacing is given by percentage of roots splitting $= 32 \cdot 14\ (S)^{0 \cdot 324}$ where $S =$ the mean number of inches of row per plant. The data do not support the common belief that splitting is encouraged by fluctuations in the water supply. It is unlikely that these plants were short of water at any period after the seedling stage and fluctuations in the soil water content are most likely to occur in the closely-spaced plots, and it is on these that the splitting is least severe. A numerical expression of the relation between spacing and bolting cannot be given because at the widest spacings, here, as with the widest spacings of the globe beet, there is a sudden increase in the incidence of bolting.

Within limits, bolting and splitting, when they do occur, tend to be progressive, increasing in amount throughout the season, and may perhaps be looked upon as results of the advancing development of the plant—modified by such factors as

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* Compared with a minimum of 2¼″ subsequently recommended by Ministry of Agriculture (Marketing Leaflet No. 102, 1948).

TABLE III.

Yield and other data for Carrots.

Thinning distance	No. of plants per foot of row	No. of plants as % theory.	Bolters as % of total.	Large roots as % total.	Split roots as % large.	Wt. large roots not split.	Wt. large roots split.	Total wt. large roots.	Total wt. of roots.	Mean wt. of large unsplit roots.	Mean wt. of large split roots.	Mean wt. of all large roots.	Mean wt. of of all roots.	Calcu-lated* total yield lb. per plot.
						lb. per plot (excluding bolters).				oz.				
Var. Early Wonder														
1 in.	8·14	68	7·2	86	35	8·7	6·3	15·0	15·3	2·19	2·90	2·58	2·34	16·4
2 in.	5·26	88	7·4	88	42	6·4	5·9	12·3	12·4	2·72	3·41	3·11	2·88	13·4
3 in.	4·14	104	7·8	91	46	5·5	5·6	11·1	11·1	2·96	3·91	3·38	3·29	12·1
4 in.	3·21	107	10·8	87	51	4·7	5·3	10·0	10·0	3·16	4·26	3·72	3·65	11·2
6 in.	2·29	115	12·6	84	53	3·5	4·4	7·8	7·8	4·17	4·99	4·60	4·47	8·9
Sig. Diff. (P = 0·05)						**1·3**	**1·7**	**1·3**	**1·5**					
Var. Altrincham														
1 in.	5·00	42	33	62	32	4·9	3·6	8·4	8·6	2·70	4·15	3·09		12·8
2 in.	3·79	63	32	66	33	4·8	3·2	7·9	8·0	3·25	4·47	3·65		11·7
3 in.	3·14	79	34	62	37	3·6	2·9	6·4	6·5	3·30	4·44	3·73		10·2
4 in.	2·64	88	32	64	35	2·9	2·4	5·3	5·4	3·31	5·27	3·99		8·4
6 in.	2·07	104	34	63	36	2·8	2·2	4·9	5·0	3·80	5·28	4·34		7·7
Sig. Diff. (P. = 0·05)						**2·0**	**1·1**	**2·3**	**2·4**					

* Yields calculated on the assumption that " bolters " had roots equal in weight to those of " non-bolters ".

season, locality, variety, and manuring. If with carrots, as was suggested for globe beet, close spacing not only reduces the size of the plant, but also retards its physiological development, so that the widely-spaced plants are physiologically older than the closely-spaced ones, then more splitting and bolting (on any given date when these phenomena are actually occurring) at the wider spacings accords with expectation. This suggestion finds some support in the fact that at each spacing the mean weight of the split roots is greater than the mean weight of the unsplit ones. The result of this effect of spacing on splitting is that whilst the total yield of large roots increases as the spacing decreases, approximately doubling as the population per foot of row increases from 2·29 to 8·14 the increase in the yield of unsplit roots is still greater (160 per cent.).

Increases in yield with increasing plant population are proportionately much greater here than with the beet and parsnips and suggest for carrots a very great dependence of yield upon population, and that if, with a poor stand of plants high yields are obtained by great growth of the individual plants this may be accompanied by much splitting. This was, in fact, found by Wallace and his co-workers (1942). In a manurial experiment they found that on certain plots where the germination was poor (and hence the stand of plants thin), the percentage of roots which split was abnormally high. It appears therefore, that the commercial practice of not thinning carrots is a sound one. Provided a good stand of plants is obtained, and the data presented here suggest that 8 plants per foot of row is not excessive, this is likely to give maximum yields of sound unsplit roots. There seems to be no justification for such thinning distances as 4 in. and 6 in. when maximum yield is desired.

Variety Altrincham. The germination of these was very poor and, later, the incidence of bolting very heavy. This is indicated by the data collected at harvest time (31st August, 1948) and assembled in Table III. As with Early Wonder carrots, the weights of bolters are not included. It will be seen that on the 1 in. plot no more than five plants per foot of row were obtained, although the differences between the actual and expected number of plants are smaller on the more widely-spaced plots. The variability in stand was so great that differences in plant density between the 2 in. and 3 in., 3 in. and 4 in., 4 in. and 6 in. plots were not significant. Conclusions based on Table III have, therefore, to be accepted with caution. Bolting, although severe, was not here affected by spacing, but inspection just before harvest suggested that nearly all potential bolters had by that date bolted, and there were very few plants in the early stages of bolting. The percentage of roots split, also, is little affected by the spacing, although the two lowest figures do occur on the 1 in., and 2 in. plots (with 5·00 and 3·79 plants per foot of row respectively). For the several spacings, however, the mean root weight for unsplit roots is always exceeded by the mean root weight for the split roots. Further, the highest value for the mean root weight of unsplit roots (3·80 oz. on the 6 in. plot) is less than the lowest mean root weight of split roots (4·15 oz. on the 1 in. plots), so that the data are not incompatible with the view that the larger and perhaps physiologically older plants are most susceptible to splitting.

As with the Early Wonder carrots, yields of large roots are very dependent on the plant population, but in all probability a denser stand of plants than the maximum obtained here (5·00 plants per foot of row) would have given higher yields than any of these obtained.

TABLE IV.

Regression coefficients for relation between spacing and growth and yield of root vegetables.

	BEET.			PARSNIP.	CARROT.	
	Crimson Globe.	Cheltenham Green Top.	Exhibition Black.	Tender and True.	Early Wonder.	Altrincham.
Range of thinning distances employed	1″-6″	1″-6″	1″-6″	2″-8″	1″-6″	1″-6″
Range of population densities obtained (No. of plants per foot of row)	2·17-5·83	2·25-6·58	2·42-6·33	1·71-4·41	2·29-8·14	2·07-5·00
Total weight of tops + roots per plot, lb. =	$5\cdot86(N)^{\cdot398}$	$4\cdot46(N)^{\cdot356}$	$3\cdot91(N)^{\cdot383}$	$12\cdot1(N)^{\cdot292}$	—	—
Total weight of roots per plot, lb. =	$6\cdot27(N)^{\cdot297}$ $*3\cdot83(N)^{\cdot415}$	$3\cdot44(N)^{\cdot324}$	$4\cdot09(N)^{\cdot300}$	$6\cdot1(N)^{\cdot345}$	$\dagger1\cdot97(N)^{\cdot449}$	$\dagger0\cdot93(N)^{\cdot626}$
Total weight of large roots per plot, lb. =	$7\cdot58(N)^{\cdot248}$ $*4\cdot15(N)^{\cdot392}$	—	—	—	$1\cdot44(N)^{\cdot499}$	$0\cdot49(N)^{\cdot681}$
Mean root weight, oz.	$1\cdot87(S)^{\cdot704}$ $*2\cdot21(S)^{\cdot590}$	$1\cdot94(S)^{\cdot660}$	$2\cdot03(S)^{\cdot698}$	$2\cdot95(S)^{\cdot644}$	$1\cdot91(S)^{\cdot504}$	$2\cdot30(S)^{\cdot366}$

* Values calculated omitting 1″ plots.

† Based on yields calculated on the assumption that bolters had roots equal in weight to non-bolters.

DISCUSSION.

Always where we have data for weights of tops as well as of roots the total yield of roots and tops falls as the number of plants per plot diminishes. At the wider spacings, weight per plant is increased but the increase never fully compensates for the reduced number of plants. With one exception (the 1 in. plots for Globe Beet), a similar relationship holds if total yields of roots only are considered. These relationships appear logarithmic and can be given numerical expression by either of two methods. The logarithm of yield may be plotted against logarithm of the number of plants per plot, using individual plot weights and numbers. By this means 25 or 30 values are available (depending on whether we have 5 or 6 replications), for calculating the regression of yield on plant number. But on individual plots yield will be influenced to a variable extent by factors other than the plant population. The alternative method is to use only the mean yields for the five spacings in deducing the regression line. This method has the disadvantage that few values are available for calculating the regression, but has the advantage that the effects of factors other than spacing are eliminated. Further, each yield value used is the mean of five or six determinations at that thinning distance and we can attach a standard error to these mean values and then reject any calculated regression line which falls outside the limits set by these standard errors. The regression coefficients shown in Table IV (with two exceptions) do all fall within these limits. We can, therefore, be quite sure of the validity of these expressions for the thinning distances employed, and under the conditions of these experiments for this year.

The two exceptions are the total yield of roots and yield of large roots for the Globe Beet, and here only by excluding the values for the 1 in. plots can we get a regression line which falls within the limits of the standard errors of the mean yields at each thinning distance. Yield per plot is given by the generalized equation, (Yield) $Y = A (N)^b$, where $N =$ the number of plants per plot. The value of b is a measure of the dependence of yield on plant population. The more nearly the value approaches unity, the more closely is yield related to plant number. The regression coefficients express concisely the relationships deduced from a consideration of the data in Tables I, II and III, and indicate clearly that whereas for Long Beet and parsnips the dependence of yield on plant population is slight, it is somewhat greater for Globe Beet (if the 1 in. plots are excluded), and still greater for carrots. Hence, within the limits of the thinning distances employed, the diminished number of plants present at the wider spacings is largely compensated for by their increased growth with parsnips and Long Beet whilst with Globe Beet the extent of this compensation is slight, and with carrots, much less.

A similar conclusion is reached, if we consider the relation between mean root weight (W) and the mean number of inches of row per plant (S). $W = A (S)^b$, a high value for b indicating that root weight is very dependent on the extent of the space allotted to the plant and that the plants at the wider spacing utilize to the full the increased available space. With carrots, where the value for b is low, the necessity of a regular stand of plants is emphasized.

The possibility exists, however, that the extent of the dependence of yield on spacing within the row is also affected by inter-row competition. As the plants are not " on the square " it may be that when inter-row competition is severe small gaps in one row are compensated for partly by increased growth of adjoining plants in the same row and partly by increased growth of plants in the adjacent rows. Presumably the optimum row distance would be one at which yield was not dependent on the regularity or density of the plants within the row. As we are concerned with yields of large roots it would thus appear that, when yields of the order obtained here are to be expected, 18 in. is about the correct inter-row distance for Long Beet and parsnips but is excessive for both Globe Beet and carrots. There is also evidence that dependence of yield on plant population decreases as the season progresses (Warne, 1951). Early in the season the yield of carrots and beet grown at several spacings is almost directly proportional to the number of plants present. Later dependence of yield on plant number is reduced, as the weight of individual plants becomes more dependent on the space available per plant.

The relations between the yields of " large " roots and plant densities are not so regular as those for total yield, and, in fact, over a wide range of thinning distances such relations do not exist for Long Beet and parsnips. This is not altogether unexpected, as the " large " roots represent only an arbitrarily chosen fraction of the total yield of roots. With both varieties of carrots however, virtually all the roots fall in the " large " category, and with Globe Beet a relation is found to exist between spacing and yield of large roots. The yield of large roots is again given by $A\,(N)^b$ and in these three cases (omitting the 1 in. plots of Globe Beet) the value of b is reasonably high, suggesting that the relation between yield of large roots and plant numbers is with 18 in. rows a close one, and presumably, therefore, not likely to be obliterated by the operation of other factors. The " theoretical yield " of large roots for individual plots can be calculated using the appropriate equation in Table IV, and compared with the actual yields obtained. When this is done for the Globe Beet (excluding the 1 in. plots) the discrepancy between the calculated yield and the actual yield, as a percentage of the actual yield, exceeds 20 per cent. only for one plot out of twenty, and attains 10 per cent. or over in only ten plots out of twenty. For the Early Wonder carrots the corresponding discrepancy reaches a level of 20 per cent. on two plots out of thirty, and attains 10 per cent. on only twelve out of the total of thirty plots. It is clear, therefore, that in these experiments local variations in soil, in the incidence of pests etc. do not upset the general relationship between the yield of large roots and the plant number for Globe Beet and carrots.

We have evidence therefore, that maximum yield is to be obtained with carrots (especially) and Globe Beet only by attaining a close and regular stand of plants. With carrots not less than eight plants per foot of row and for Globe Beet not less than 4·2 plants per foot of row appear to be desirable. An even denser stand of plants (5·8 per foot of row) did not reduce the yield of Globe Beet significantly. Although this corresponds to a mean distance of 2·1 in. it represents the stand of plants obtained by adopting a nominal 1 in. thinning distance which may be compared with commonly recommended distances of 3, 4 or even 6 in. Long Beet and parsnip in their growth respond much more than do carrots to an increase in the available space, and this is in agreement with the independence of the yield of large roots and plant density, so that varying the thinning distance from 1 to 4 in. (giving 6·6 to 2·9 plants per foot of row) for Long Beet, and from 2 in. to 6 in. (4·4 to 2·0 plants per foot of row) for parsnips does not significantly affect the yield of large roots. With densities less than these the yield drops. These results are contrary to the impression gained on handling the crops at harvest. Always on the closely-spaced plots the high number and proportion of small roots leaves one with a very poor impression of the crop, although it is not the number of small roots, but the number and weight of the large roots which is important in assessing yield.

When the yield is very dependent on the plant population, the difficulties which may arise in comparing the yields of several varieties become apparent. The plots in these experiments serve to indicate the difficulty of obtaining identical stands of plants of any two or more varieties. With beet, varietal differences in plant density are small, but even here in one case a difference of 22 per cent. occurs (4·25 plants per foot for Crimson Globe, and 5·17 for Cheltenham Greentop). A difference of this magnitude at this level could, for Crimson Globe beet, alter the yield of large roots, by 8 per cent. For the carrots the poor germination of one variety caused such differences in the stand of plants that comparison of yield would be impossible if one thinning distance only were employed. Further, the two varieties of carrot appear to respond differently to change in thinning. These data, therefore, emphasize the desirability of carrying out variety trials at several thinning distances, so that, for each variety, a regression of yield on plant density may be obtained.

SUMMARY.

1. Carrots (2 varieties), Beet (3 varieties) and Parsnips (1 variety) were grown at a uniform inter-row distance, and at five thinning distances.

2. The thinning distances employed gave plant populations (per foot of row) varying from approximately 2 to 6 for beet, 1·7 to 4·4 for parsnips and 2·3 to 8·0 for one variety and 2·0 to 5·0 for the other variety of carrots. At the closer spacings the population of plants always fell far short of the expected number.

3. Within these ranges of population densities, total yields of tops + roots (when top weights were obtained), and total yield of roots always increased as the number of plants per plot increased (except for the total yield of roots with one spacing of Globe Beet). Mean plant weight and mean root weight always increased as the space allotted to each plant increased.

4. Examination of the regression coefficients suggests that the yield of carrots is more dependent on the plant population than is the yield of beet and parsnips. The maximum yield of "large" carrots was obtained with the closest spacings. With Globe Beet, populations of 4·2 and 5·8 per foot of row gave higher yields of "large" roots than were obtained with sparser stands of plants. Yields of "large" Long Beets were not altered significantly by changes in the plant density from 6·5 to 3·0 per foot. With one variety the yield fell when the number of plants per foot of row was further reduced to 2·2. Yields of "large" parsnips were not affected by changes in the number of plants per foot of row between 2·0 and 4·4 but fell with a further reduction in the number of plants. Generally, maximum yields were obtained with spacings much closer than those usually recommended.

5. With Globe Beet and one variety of carrots, the incidence of bolting was decreased by close spacing of the plants.

6. With one variety of carrots (but not with the other), close spacing reduced the percentage of roots splitting.

ACKNOWLEDGMENTS.

I wish to thank Professor Ashby for placing at my disposal the facilities for this work. The cultural operations were all efficiently supervised by Mr. F. Wallen.

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(*Received* 22/2/50.)

THE VARIABILITY OF APPLE TREES

II. THE OPTIMUM SIZE FOR UNGUARDED PLOTS

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THERE has long been a difference of opinion whether the experimental unit with apples should be one tree or many. The subject is here studied for treatments that can be applied to one tree without affecting its neighbours.

INTRODUCTION.

Statement of the problem.

When designing a field trial with apples a decision has to be made whether to use many plots of few trees or few plots of many trees. Much work has been published on this subject and this will be reviewed, followed by further work on lines recently suggested (11). Only trials that do not need guard rows will be here considered.

Review of previous work.

As Hoblyn (6) has pointed out, crops, trunk girths etc. are necessarily measured on individual trees and he therefore recommended the use of one-tree plots. On the other hand, Kemmer (7) drew attention to the danger of losing trees from winter frosts and thought it desirable to use plots of at least three trees. This applies, of course, only to localities where the winters are very cold ; but other factors, such as disease, may also be responsible for the loss or severe injury of individual trees, in which event the same consideration would apply.

The first uniformity trial with apples was that of Batchelor and Reed (1), who concluded that there is " little gained in accuracy by increasing the plot to include more than eight adjacent trees ". Strickland (13) thought plots of four to eight trees " very convenient ". Both these bodies of data will be examined further below.

As Desaymard (5) and Massibot (8) have both pointed out, the standard error should be small in relation to the mean. For this reason they thought that plots of apples on seedling rootstocks should contain three or four trees, but that one-tree plots could be used with clonal material. Their objection to variable plots is, of course, quite justified, though recent work (10) suggests that they were mistaken in the figures they assumed for the coefficient of variation, at least for English conditions.

The purpose of the present paper.

It is intended in this paper :

(*a*) To re-examine the data of Batchelor and Reed (1) and of Strickland (13) in the light of recent suggestions (11) that in treating uniformity data: (i), the number of plots to a block should be standardized and (ii), attention should be given to the economizing of land.

(*b*) To examine some unpublished data from East Malling.

(*c*) To collate the various recommendations made here and elsewhere concerning plot size so as to arrive at a soundly based conclusion.

Methods to be used.

These problems could have been approached using the method of Fairfield Smith (12), who showed empirically that if Var_x is the variance between plots of x units and Var_1 is the variance between the units themselves, then

$$\log Var_x = \log Var_1 - b \log x,$$

where b is a constant between 0 and 1. He assumed that the cost of a plot was represented by a linear relationship:

$$K_1 + K_2 x,$$

and concluded that the most economical size of plot is one containing units to the number of $\dfrac{b K_1}{(1-b)\ K_2}$.

It follows that if the cost of an experiment is taken to depend entirely upon the total area, $K_1 = 0$ and the smallest possible plot should be used. If, on the other hand, the cost is determined solely by the number of plots, $K_2 = 0$ and plots should be as large as possible. It remains, however, to be shown that in any instance plot size is of practical importance; because, for some values of b, plots far from the ideal can be used very effectively.

This analysis is very well based and does suggest an alternative method to the one employed. In this, coefficients of variation are worked out so as to evaluate the number of trees needed to make a given difference significant. The amount of computing is probably about the same whichever method is adopted and it appears to the writers preferable actually to work out the quantities to be discussed rather than to estimate them by an empirical law, however well established. Also, they prefer to avoid any assumptions about the effect of plot shape. As will be seen, the results obtained were in no way discordant with Fairfield Smith's theory, if b is assumed not to be small.

Explanation of tables.

Throughout this paper results will be presented in two ways:

(*a*) as a coefficient of variation (V), that is to say, as the standard error of the crop of plots within blocks expressed as a percentage of the mean value of the plots. In one trial the data represented trunk girth, which is better dealt with statistically as its logarithm (9). For this trial s will be given instead of V. To obtain this quantity, the logarithm of trunk girth will be added for the trees of each plot and the standard error (s) of these sums within blocks computed.

(*b*) as N, the number of trees needed to show a significant difference equal to 20 per cent. of the mean. Following Christensen (2) the number of replications needed has been taken to equal $0{\cdot}022V^2$, and N is plainly this quantity multiplied by the number of trees to a plot. In the above formula t^2 is assumed to equal $4{\cdot}4$. If the number of replicates needed for comparison is small, e.g. two, this assumption would not hold, but the approximation gives a good indication of the information obtained per tree, which is all that is necessary here. This apparent difficulty arises from the choice of a constant figure of 20 per cent. as the difference to be shown significant.

Where the data represented trunk girths a different formula was adopted. A difference of 20 per cent. corresponds approximately to a difference of 0·08 in the logarithm and this leads to the relationship:

$$N = \frac{1375\, s^2}{p}.$$

A definition has already been given of s, while p represents the number of trees to a plot.

RESULTS OF UNIFORMITY TRIALS.

Yields of Jonathan apples in Utah.

These individual tree yields were published by Batchelor and Reed (1) and are set out in their Table VI. Of the 224 figures given, those from the outside rows were omitted as being obviously different from the rest. Data from Row 5, Trees 49 and 55, were omitted also, because their values might well have been arrived at by the authors' method of fitting missing values. First, the variation within sets of twelve trees was examined but the results led to no clear conclusions, probably because of the rectangular plant, so sets of 16 trees were substituted even though this meant wasting many of the data. In this way, every plot or block taken along the rows could be matched by one taken across them. The actual sixteen-tree sets used are shown in Fig 1(*a*), the four adjacent rows in which they are situated having been chosen at random.

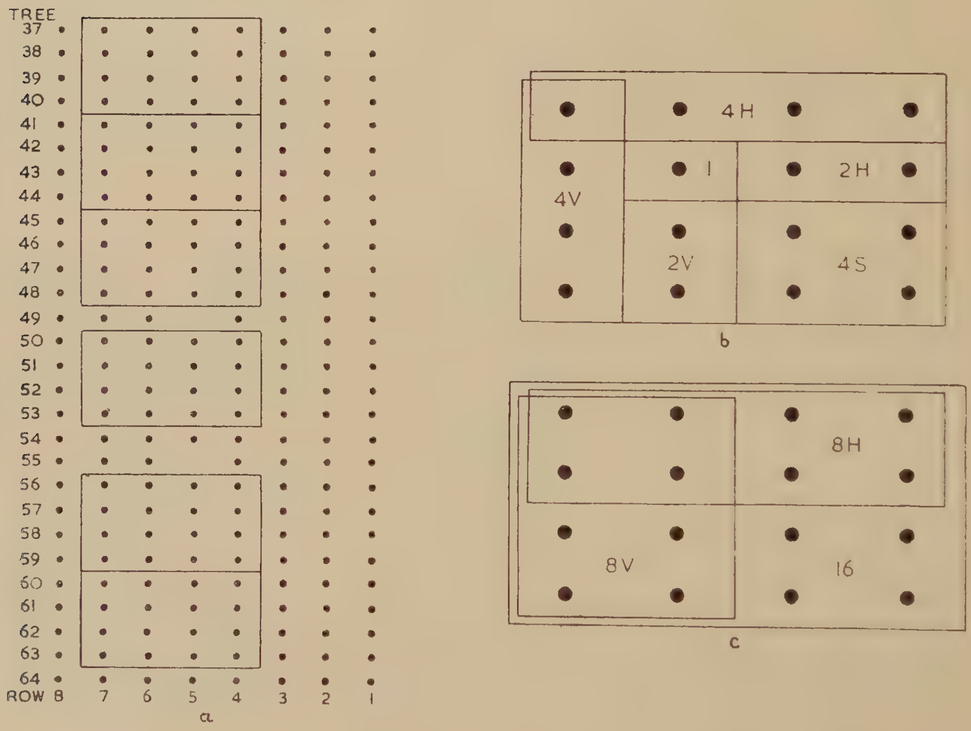

FIG. 1.

Method of allocating the apple trees of Batchelor and Reed (1918) to sets and of sub-dividing the sets. The trees were planted 30 ft. by 16 ft.

Fig. 1(*b* and *c*) illustrates the various sizes and shapes of the plots and blocks used. The plots varied in size from one tree to eight and were arranged either in rectangles in a horizontal (H) or vertical (*V*) direction, though some were in the form of a square (S). The blocks varied in size from 2 to 16 trees, according to the number of treatments and the size of plot, and were arranged in a similar way to the plots. In Table I the results are listed for experiments having two, four and eight hypothetical treatments. Each particular size and shape of plot or block is indicated by a number and a letter, thus, 2H means two trees horizontally placed see (Fig. 1(*b*)), 4S means four trees on the square (Fig. 1(*b*)) and 8V means eight trees, four by two, in a vertical rectangle (Fig. 1(*c*)). The notation, 2H : 4S, represents two treatments on plots of two trees placed horizontally arranged in a square block of four trees ; 2V : 8H, four treatments on plots of two trees vertically placed in horizontal blocks of eight trees, four by two, and so on.

TABLE I.

Table showing V, coefficient of variation, and N, the number of trees per treatment, to show a 20 per cent. difference, as calculated from the data of Batchelor and Reed.

Plot.	Block.	*V.*	*N.*
Two Treatments.			
1	2H	37·2	30·4
1	2V	33·9	25·3
2H	4S	24·2	25·8
2H	4H	25·7	29·0
2V	4S	28·6	36·0
2V	4V	20·2	18·0
4S	8H	13·0	14·8
4S	8V	10·6	10·0
4H	8H	16·1	22·8
4V	8V	25·2	56·0
8H	16S	14·2	35·2
8V	16S	17·8	56·0
Four Treatments.			
1	4S	36·3	29·0
1	4H	37·0	30·1
1	4V	32·2	22·8
2H	8H	24·8	27·0
2H	8V	23·2	23·6
2V	8H	27·8	34·0
2V	8V	26·4	30·6
4S	16S	19·0	31·6
4H	16S	17·5	26·8
4V	16S	25·2	56·0
Eight Treatments.			
1	8H	36·3	29·0
1	8V	35·4	27·6
2H	16S	25·3	28·2
2V	16S	27·9	34·2

For description of plots and blocks see Fig. 1.

It is clear from Table I that the trees were very variable. It appears, also, that variability is least for any type of block (the number of treatments being constant) when the plots extend across as *many* rows as possible. For example, the combination 2H : 4S is better than 2V : 4S, while 4H : 16S is better than 4S : 16S, which in turn is an improvement upon 4V : 16S ; though 4S : 8H and 4H : 8H provide an exception. Also, for any kind of plot, blocks are best when they extend across as *few* rows as possible. Thus, 4S : 8V gives less variability than 4S : 8H and so on for all available comparisons. It is doubtless this effect that caused the difficulty in interpreting results from sets of twelve trees and may well be a consequence of the different planting distances between and within rows.

Plot size was next considered using two methods. First of all, Table I was rearranged to form Table IA in which the results are presented in nine groups, such that the arrangement of plots within blocks is the same within each group. It is clear from this table that, within groups, V was generally less for the larger plots. For N the results were not conclusive, though in general fewer trees were needed when plots were small, this effect being most marked in those groups in which plots and blocks severally extended over the same number of rows. Thus in Groups I and VI N was reduced from 56 for four-tree plots to approximately 30 for one-tree plots, in Group III from 56 for eight-tree plots to 29 for two-tree plots, while there was a similar but smaller effect in Group VIII. The other method was to take the averages of the groups of figures shown by the lines in Table I. This is quite reasonable because, for example, every occurrence of 4H is balanced by an occurrence of 4V and so on. The results were the same as before.

TABLE IA.

The results of Table I in a new arrangement.

Group.	Plot.	Block.	*V.*	*N.*
I	1	2H	37·2	30·4
	2V	4S	28·6	36·0
	4V	8V	25·2	56·0
II	1	2V	33·9	25·3
	2H	4S	24·2	25·8
	4H	8H	16·1	22·8
III	2H	4H	25·7	29·0
	4S	8H	13·0	14·8
	8V	16S	17·8	56·0
IV	2V	4V	20·2	18·0
	4S	8V	10·6	10·0
	8H	16S	14·2	35·2
V	1	4S	36·3	29·0
	2H	8H	24·8	27·0
	2V	8V	26·4	30·6
	4S	16S	19·0	31·6
VI	1	4H	37·0	30·1
	2V	8H	27·8	34·0
	4V	16S	25·2	56·0
VII	1	4V	32·2	22·8
	2H	8V	23·2	23·6
	4H	16S	17·5	26·8
VIII	1	8H	36·3	29·0
	2V	16S	27·9	34·2
IX	1	8V	35·4	27·6
	2H	16S	25·3	28·2

For description of plots and blocks see Fig. 1 (*b* and *c*).

It follows that large plots give more information per replicate than small ones, but are probably less economical of land.

Using one-tree plots in a Latin square, V was 31·3 and N 21·6, figures that are somewhat better than those for randomized blocks.

Yields of Jonathan apples in Victoria, Australia.

These yields in one season from very uniform trees without interplanted pollinators and probably all on Northern Spy rootstock were published by Strickland (13). Fig. 2(*a*) shows how eight sets of sixteen trees were taken while Fig. 2(*b*) and (*c*) show how they were subdivided. Such choice as there was in the location of the sets was exercised at random.

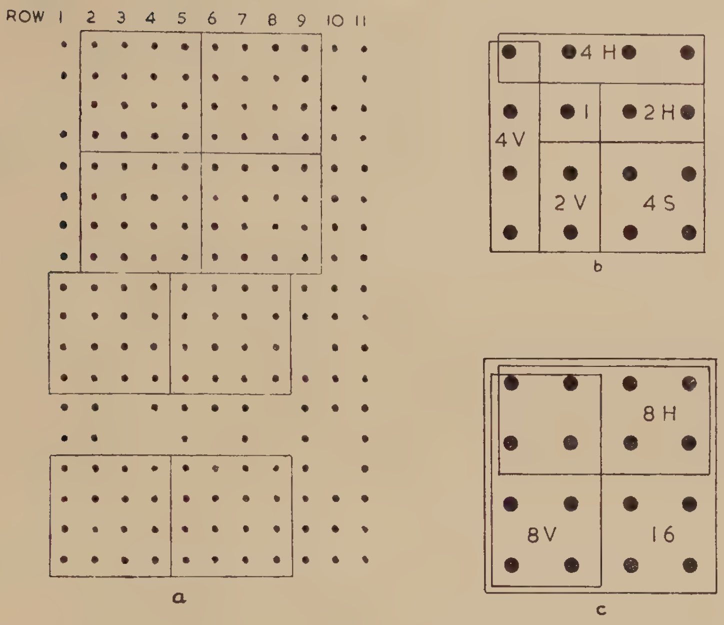
ROW 1 2 3 4 5 6 7 8 9 10 11
a
4 H
1
2 H
4 V
2 V
4 S
b
8 H
8 V
16
c

FIG. 2.

Method of allocating the apple trees of Strickland (1935) to sets and of subdividing the sets.

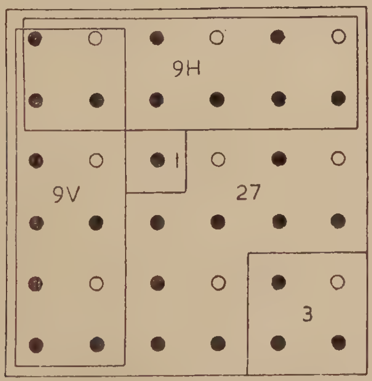

FIG. 3.

Method of subdividing a set of the Bramley's Seedling apple at East Malling. The trees were planted 24 ft. square. Rings represent pollinators.

Table II confirms Strickland's conclusion that V falls off as plot size increases, at least up to eight trees.

TABLE II.

Table showing V, coefficient of variation, and N, the number of trees per treatment, to show a 20 per cent. difference, as calculated from Strickland's crop data.

Plot.	Block.	V.	N.
Two Treatments.			
1	2H	17·3	6·5
1	2V	20·0	8·8
2H	4S	15·1	10·0
2H	4H	16·2	11·6
2V	4S	11·3	5·6
2V	4V	16·5	12·1
4S	8H	14·3	17·9
4S	8V	14·2	17·8
4H	8H	13·0	14·8
4V	8V	7·5	4·9
8H	16S	9·8	16·9
8V	16S	9·8	17·0
Four Treatments.			
1	4S	18·7	7·7
1	4H	19·3	8·2
1	4V	21·2	9·9
2H	8H	17·0	12·7
2H	8V	16·9	12·6
2V	8H	14·8	9·7
2V	8V	14·8	9·7
4S	16S	14·1	17·6
4H	16S	13·3	15·5
4V	16S	10·1	9·0
Eight Treatments.			
1	8H	20·4	9·2
1	8V	20·4	9·2
2H	16S	17·4	13·4
2V	16S	15·6	10·8

For description of plots and blocks see Fig 2.

Consequently, the larger plots give more information per replicate. On the other hand, N increases as plot size becomes larger, so the smaller plots give more information per tree. With apples this is often the more important consideration. Variability depends more upon plot size than upon the number of plots to a block. Also, there is reason for believing that long plots are better than square ones, but that blocks are better when square.

Using rows (4H) and columns (4V) within sets (16S), Latin squares could be formed. In this case V was found to be 19·0 making N equal to 8·0. Comparing this with randomized blocks of four treatments (1 : 4H, 1 : 4V and 1 : 4S) it is evident that no marked improvement had been effected.

Yields of Bramley's Seedling at East Malling.

The data available were the crops of 216 trees of Bramley's Seedling upon a mixture of rootstocks, most of which were M.II, in the periods, 1941-44 and 1945-48. These trees were 27 years old in 1941. Alternate trees in alternate rows were Grenadier pollinators. After the rejection of one tree as being younger than the rest, seven sets were formed of the kind shown in Fig. 3, these sets being sub-divided as in that diagram in horizontal (H), vertical (V) or square (S) plots and blocks as before. In this instance, however, the pollinators were not used, so that the numbers of trees per plot or block were 1, 3, 9 or 27.

The results are set out in Table III for each period separately and for the second period after an adjustment by covariance upon crops in the first. With these data also V decreased and N increased as plot size became larger, leading to the same conclusions as before. There is here a slight suggestion that variability is greater when the number of plots to a block is increased. The level of variability was markedly less in the second period than in the first and the adjustment by covariance was not very successful in reducing variability.

TABLE III.

Table showing V, coefficient of variation, and N, the number of trees per treatment to show a 20 per cent. difference as calculated from some East Malling crop data.

		1941-44.		1945-48.		1945-48 adjusted.	
Plot.	Block.	*V.*	*N.*	*V.*	*N.*	*V.*	*N.*
Three Treatments.							
1	3S	21·9	10·6	14·7	4·8	12·2	3·3
3S	9H	14·8	14·4	9·5	6·0	9·0	5·4
3S	9V	14·3	13·5	11·5	8·7	10·4	7·2
9H	27S	10·2	20·7	8·0	12·6	7·3	10·8
9V	27S	10·9	23·4	4·7	4·5	4·5	3·6
Nine Treatments.							
1	9H	22·8	11·4	15·2	5·1	13·1	3·8
1	9V	22·6	11·2	16·2	5·8	13·8	4·2
3S	27S	15·5	15·9	10·8	7·8	9·9	6·6

For description of plots and blocks see Fig. 3.

TABLE IV.

Table showing s, the standard error of the logarithm of trunk girth, and N, the number of trees per treatment to show a difference of 0·08 in the logarithm of trunk girth, corresponding approximately to a 20 per cent. difference in trunk girth. Figures calculated from East Malling data. All values of s multiplied by 1,000.

Plot.	Block.	*S.*	*N.*
Three Treatments.			
1	3	56	4·3
2	6	82	4·7
4	12	127	5·6
8	24	183	5·8

For description of plots and blocks see Fig. 4.

Plot.	Block.	*S.*	*N.*
Six Treatments.			
1	6	55	4·1
2	12	82	4·6
4	24	125	5·4
Twelve Treatments.			
1	12	55	4·1
2	24	82	4·6

Trunk girth of Cox's Orange Pippin at East Malling.

The data represented the trunk girths measured in June, 1943, of 910 trees of Cox's Orange Pippin on rootstock, M.IX, most of them planted during the winter of 1935-36. Every fourth row was made up of Worcester Pearmain pollinators also on M.IX. After the rejection of younger trees eighteen 24-tree sets of Cox's were formed, each being subdivided as shown in Fig. 4.

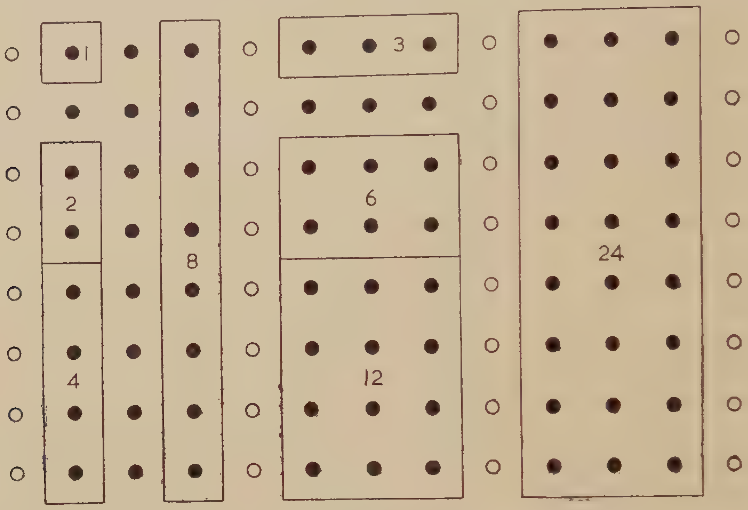

FIG. 4.

Method of subdividing a set of the Cox's Orange Pippin apple at East Malling. The trees were planted 12 ft. square. Rings represent pollinators.

Two, three, six and twelve treatments were considered using the actual girths in logarithmic transformation. For two treatments the results were complicated by the varying relations between plot and block shape and are not shown in Table IV. They are, however, in general agreement with the rest, which are set out in Table IV. The trees were originally planted commercially and were not, therefore distributed at random but possibly in the order in which they were grown in the nursery. An attempt was made to adjust for this by use of covariance upon the logarithm of trunk girth in June, 1937, but little correlation was found so this effect can hardly be serious.

Variability plainly depends upon plot size rather than upon the number of plots to a block. The third column of Table IV refers not to V but to s, so should be divided by the number of trees to a plot to indicate the information per replicate. When this is done, it appears that large plots are better. As usual, N is smaller for the smaller plot sizes, thus showing that more information is obtained per tree by using small plots.

DISCUSSION.

The above results are based upon trials from three continents and may therefore be taken to have very general application.

In the main, variability depended upon plot size rather than the number of plots to a block. It follows that efforts to reduce variability by experimental lay-outs aimed at reducing block size, such as confounding and the use of incomplete block designs, are likely to prove unavailing.

On the other hand, there is some evidence that plots should be long and narrow and blocks square and compact, a result which is rather surprising in view of this apparent unimportance of plot dimensions. For the data of Batchelor and Reed, variability lay chiefly between rows and this had important consequences on the best shape for plots and blocks. These results agree with those of Christidis (3, 4). The variation between rows in this instance is probably due to the distance between them, but with annual species it might well arise from irregular drilling.

Where Latin squares could be used, they showed little improvement on randomized blocks.

As Desaymard (5) and Massibot (8) have pointed out, plots should not be so small that V is unreasonably large. Since a value of V greater than 30 may give rise to considerable non-normality of data, this figure may be taken to represent the upper limit ; and it is known that one-tree plots can often be unsatisfactory in this way (10), at least in respect of cropping during a short period. Nevertheless they may be used if it is intended to work only with quantities that give lower variability, such as crop over a period of several years or increment in the logarithm of trunk girth.

The objection of Kemmer (7) to one-tree plots should also be considered. It is certainly unwise to use such small plots when there is a danger of losing many trees ; and disease may cause this as well as cold winters. Of course, a few casualties can be dealt with by the ordinary missing plot technique, but where many gaps are to be expected larger plots should be used.

For apples the authors recommend the use of one-tree plots, unless there are good reasons to the contrary, because they give the most information *per tree* and are thus most economical in ground and farm labour, two big considerations. Further, since recording labour with trees depends upon the number of trees rather than the number of plots, economy is effected here also. These considerations may not be dominant in every trial, while the effect of introducing guard rows between plots will be discussed in a future paper ; but this investigation strongly supports the recommendation made by Hoblyn (6) nearly twenty years ago to use one-tree plots, at least as a general rule.

From the tables it is clear that variability is by no means constant from trial to trial and consequently no general recommendation can be made as to the number of replicates required. In any event this must depend also upon the accuracy being sought. Usually, eight or ten replicates of one-tree plots are needed to show a difference of 20 per cent. in crop, though the values of N have reached as much as 30·4. Examination of Table III shows how the same trial may give very different results in different periods.

SUMMARY.

A study is made of optimum plot size for apple experiments with no guard rows on the basis of four sets of uniformity data. It is concluded that larger plots give more information per replicate, but smaller ones give more information per tree and this is taken to be the more important consideration. Objections have been made in

the past to one-tree plots on the grounds that they (*a*) are too variable and (*b*) give rise to too many missing plots. Either objection may have substance in particular instances, but as a general rule one-tree plots are to be recommended.

ACKNOWLEDGMENT.

The writers wish to thank Dr. E. R. Parker of the Citrus Experiment Station, Riverside, California, for supplying information about the trees used by Batchelor and Reed.

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(*Received* 5/4/50.)

THE CONTROL OF "WHIPTAIL" IN BROCCOLI AND CAULIFLOWER

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THE condition known as " whiptail " in cauliflower and broccoli has been shown to be due to a deficiency of molybdenum (Davies, 1945). The symptoms of this deficiency disease when produced in sand culture have been described by Hewitt and Jones (1947) and, in the later stages of development, resemble the condition found in the field.

In England and Wales the control of whiptail has recently been reported by Jones (1950) and Plant (1950) and the following account is a record of experiments and observations made from this station since 1947. The earliest record of this problem in England appears to have been made by Ogilvie and Hickman (1936) and, as a consequence of an expanding vegetable acreage in this country during and since the second World War, the incidence of whiptail has undoubtedly increased. The reason for this may be largely connected with the use of broken-up pasture for cauliflower and broccoli growing. In cases where liming, shown by soil tests to be necessary, has been omitted or imperfectly carried out, the deficiency has frequently occurred. Investigation of the soil conditions causing whiptail in the field is complicated by the fact that the soils are generally acid and the plants are affected by injurious acidity factors, notably by excess of manganese.

VISUAL SYMPTOMS.

The syndrome of whiptail is easily recognized in the field but there is considerable variation among individual plants according to the severity of the deficiency. In Table 1 the three main types of whiptail specimens are tabulated (Figs. 1-6). Types 2 and 3 occur more frequently in cauliflower whilst all types are common in broccoli.

The chief symptom entails a reduction in the amount of lamina, which usually has irregular and wavy margins. The lamina may be markedly thick and succulent (Fig. 4) or convoluted (Fig. 6). Leaf colour is not generally affected in whiptail but

TABLE I.

Description of whiptail symptoms.

Type.	Habit.	Lamina shape.	Petiole.	Growing point.	Hypocotyl.	Figs.
1	Upright	irregular outline	medium length	stub-like or stunted	bud proliferation	1, 3
2	Branched	irregular	medium length	stub-like or stunted	bud proliferation	5
3	Rosette	irregular, wavy, puckered	elongated	Stub-like or blind	bud proliferation	2, 4, 6

some specimens are noticeably blue-green. Mid-ribs may be almost bare and when completely devoid of laminae are typically whiptail in appearance (Fig. 1). Growing points are stunted, curds fail to develop and, where the deficiency is progressive, meristematic differentiation ceases and the plants develop a dome-like centre (Fig. 4). In this condition the plant appears blind but an inspection of the subterranean parts frequently reveals bud proliferation from the hypocotyl (Fig. 5).

INCIDENCE OF THE DEFICIENCY.

The deficiency has been found on a wide range of acid soils derived from differing parent materials, including Granite, Devonian (shale), Old Red Sandstone, Keuper and Lower Greensand. As the crops are grown in special market-garden areas or suitable farming localities, the range of parent material investigated has been limited by the geological formations and drifts occurring in these districts. It appears that parent material has little effect on the incidence of the deficiency but soil acidity is important. Whiptail, however, does not occur on every acid soil.

Whilst systematic trials on the susceptibility of varieties to whiptail have not been carried out, the following varieties have been observed to be affected :

Cauliflower : Majestic, Novo, Veitch's Autumn Giant.

Broccoli : Roscoff types, Early Feltham, Satisfaction, St. George.

DIAGNOSTIC TECHNIQUES.

Visual.

A description of whiptail types has already been given (Table I, Figs. 1-6). The distribution of the deficiency in affected fields follows the trend of the soil pH, and in areas where this falls below 5·5 to 6·0, whiptail usually occurs. Occasionally, a permanent pasture with a low pH (4·5) is ploughed and cropped with early potatoes ; if followed by broccoli the plants may be so badly affected that few healthy plants can be found.

Chemical.

Nitrate test. It has been shown by Hewitt and Jones (1948) that whiptail plants accumulate nitrates. A series of tissue-tests carried out on broccoli varieties, using a modification of Noll's (1945) method, gave the following results (Table II).

TABLE II.

Nitrate content in p.p.m. in fresh leaf mid-rib. Leaves sampled 22.2.49.

Variety.	Healthy leaf.	Whiptail leaf.	Remarks.
D.K.7	246	282	no change
Harding's Roscoff	176	528	increase
Early Feltham	176	600	increase
Satisfaction	246	176	decrease
A.6.	264	246	no change
Superb Early White	141	246	increase
St. George	176	176	no change
Roscoff No. 2	246	528	increase
Fairweather's Early	282	422	increase

PLATE I

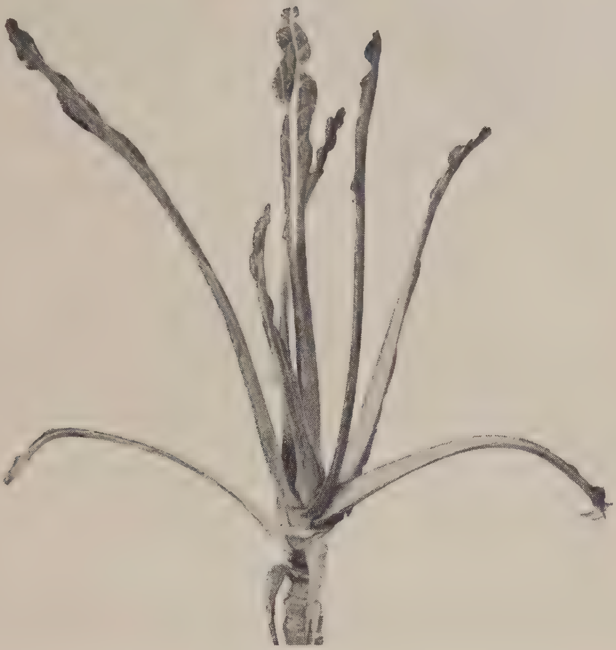

FIG. 1.

Broccoli. "Whiptail." Upright habit. Marked suppression of lamina.

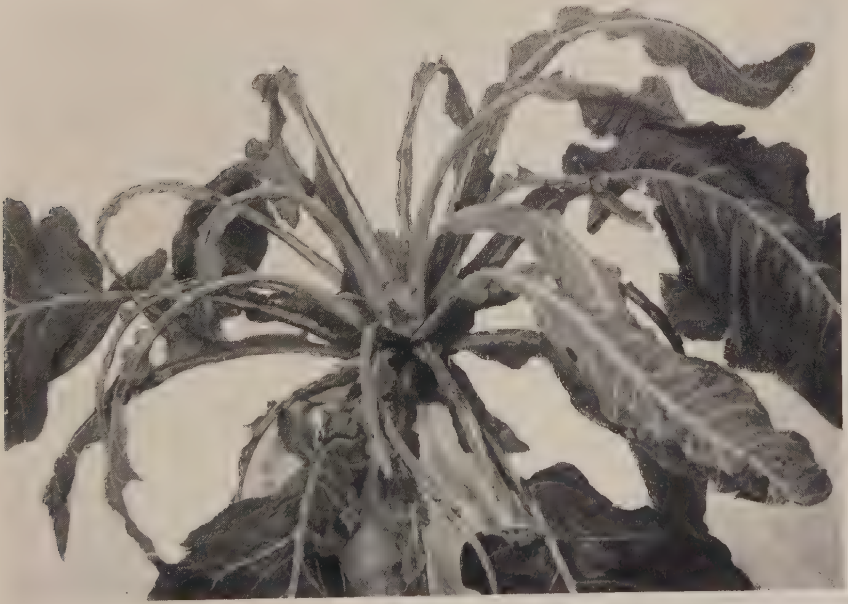

FIG. 2.

Broccoli. "Whiptail." Rosette habit. Stub-like growing point.

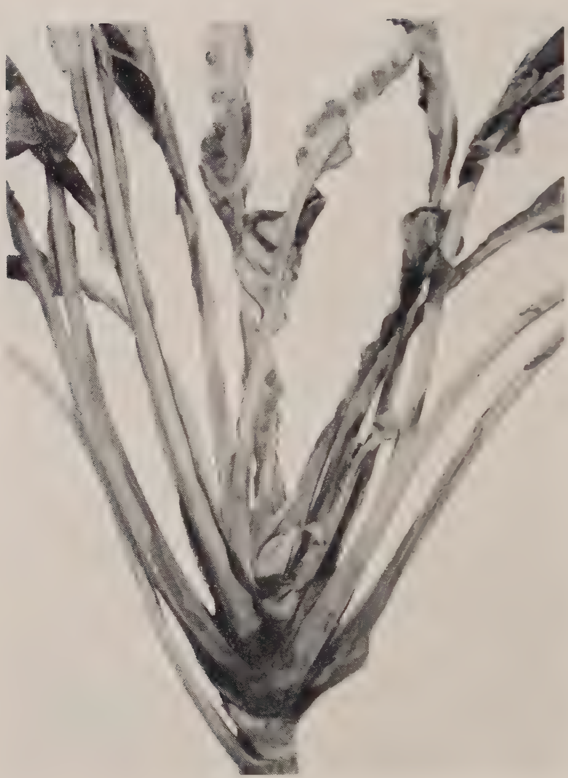

FIG. 3.
Cauliflower. "Whiptail." Stub-like centre. Irregular lamina.

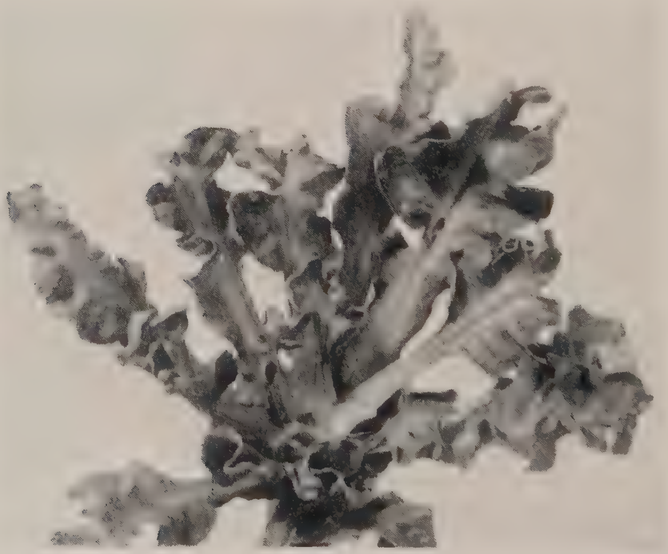

FIG. 4.
Broccoli. "Whiptail." "Dome-like" centre. Succulent foliage.

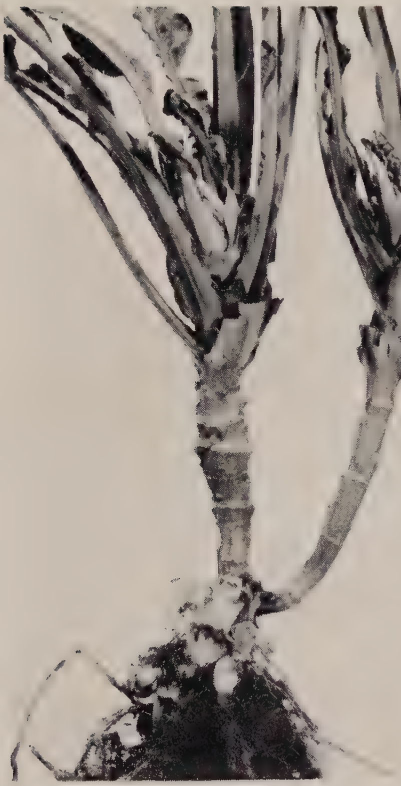

FIG. 5.

Cauliflower. "Whiptail." Branched. Bud proliferation at hypocotyl.

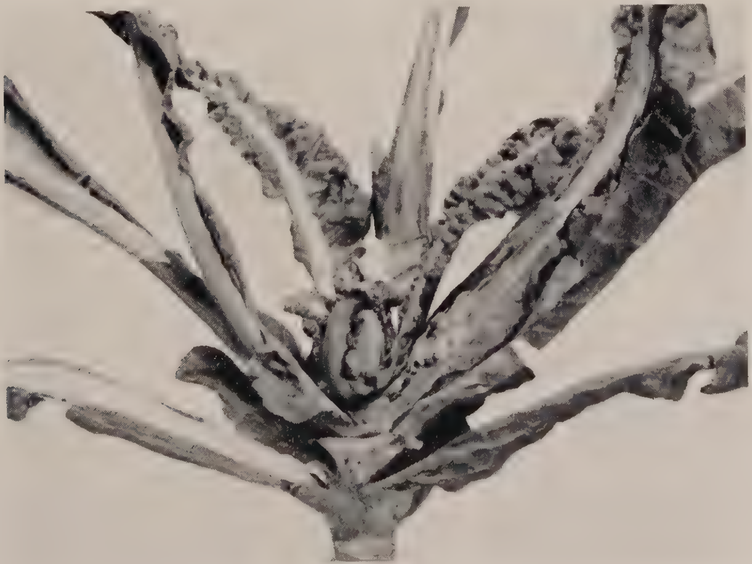

FIG. 6.

Cauliflower. "Whiptail." Lamina succulent and convoluted.

Thus, 5 out of 9 varieties showed higher nitrate contents in the whiptail condition; 3 showed the same value and 1 gave a lower figure. It would appear from these results that the test under field conditions is not specific. Moreover, it may be noted that the nitrate content of the leaf mid-ribs in relation to their age and development may show a variation from 150 p.p.m. nitrate in the young leaves to 1300 p.p.m. nitrate in the oldest ones.

Molybdenum content. During the period 1948-49 it has been possible to compare the molybdenum contents of whiptail and healthy plants occurring together in a number of fields. In Table III, the molybdenum values are given for plants grown on different soils, using the method of Piper and Beckworth (1948).

TABLE III.

The molybdenum content of healthy and whiptail broccoli and cauliflowers.

Locality.	Parent Material.	Crop.	Leaves Sampled.	Mo content p.p.m. D.M.	
				Healthy.	Whiptail.
Cornwall	Devonian	Broc.	Feb. 1948	1·80	0·08
S.W. Wales	Old Red Sandstone	Broc.	Nov. 1948	0·15	0·03
The Gower, Glamorgan, S. Wales	Old Red Sandstone and Carboniferous Limestone drift	Caul.	Nov. 1948	0·25	0·07
Bromham, Wilts.	Lower Greensand	Caul.	Nov. 1949	0·19	0·02

Although it is evident that the molybdenum contents of the whiptail leaves are less than those of the healthy ones, the molybdenum range within which whiptail leaves are found varies considerably. Sampling error is no doubt partly responsible for this as the molybdenum content of whiptail leaves can vary from 0·58 p.p.m. in the youngest to 0·02 p.p.m. in the outer leaves.

FIELD EXPERIMENTS ON BROCCOLI.

Four Centres in Cornwall were used.

Centres.	Year.	Soil.	pH.	Remarks.
A. Ludgvan	1948-49	Devonian	4·7-5·0	Whiptail in broccoli, 1947-48
B. Bussow	1948-49	Granite	4·2-4·5	Permanent pasture, 1948
C. Kehelland ..	1949-50	Devonian	4·7-5·0	Whiptail reported on farm
D. Budock	1949-50	Devonian	5·2-5·5	Whiptail reported on farm

EXPERIMENTAL LAY-OUT.

At centres A and B treatments were replicated threefold; at Centres C and D the design was in the form of a Latin Square. The treatments were as follows:

Centres A, B. Broccoli planted out at the end of July, 1948.

1. Control.
2. Ground limestone three tons per acre. Applied 25th June, 1948.
3. Gypsum 3 tons per acre. Applied 25th June, 1948.
4. Ammonium molybdate 4 lb. per acre (sand as carrier). Applied 25th June, 1948.
5. Ammonium molybdate 8 lb. per acre (sand as carrier). Applied 25th June, 1948.
6. Sand alone. Applied 25th June, 1948.
7. Ammonium molybdate foliage spray (100 p.p.m.) 2 applications. Applied 20th August, 1948 and 10th September, 1948.

Centres C, D. Broccoli planted out at the end of July, 1949.

1. Control.
2. Ground limestone 3 tons per acre. Applied 21st June, 1949.
3. Sodium molybdate 2 lb. per acre. Applied 21st June, 1949.
4. Sodium molybdate 4 lb. per acre. Applied 21st June, 1949.

A base dressing consisting of muriate of potash and ammonium phosphate was given (5 cwt. per acre) to Centres A, B, C and D.

Molybdenum content of ground limestone and gypsum.

Examination of the ground limestone and gypsum for total and available molybdenum gave the following results :

	Total Mo content (p.p.m.)	Water-soluble Mo content (p.p.m.)	Mo supplied at 3 tons per acre (gm.)
Ground limestone ..	0·40	0·20	0·4
Gypsum	0·10	0·10	0·2

It is considered unlikely that the amount of molybdenum supplied by limestone or gypsum would seriously affect the results of these treatments. The minimum requirement of a crop of broccoli or cauliflower is about 5·0 gm. Sodium molybdate at 2 lb. per acre supplied approximately 450 gm. of molybdenum.

Leaf sampling.

For all samples, leaves midway between the inside and outside were taken. The mid-rib only was used for the nitrate test, the lamina being cut away and discarded.

Results.

Yields. At Centre A, the yields of plots were obtained (Table IV). At Centres C and D the percentages of whiptail and numbers of marketable heads, prior to harvesting, were used. (Table V.)

The yields at Centre B were not recorded because the experiment failed to show whiptail and because growth in all plots, irrespective of treatment, was good.

TABLE IV.

Centre A. Mean yields, in tons per acre, of untrimmed heads.

Treatment.							
Control.	Ground Limestone.	Gypsum.	Ammonium molybdate 4 lb.	Ammonium molybdate 8 lb.	Sand alone.	Ammonium molybdate spray.	Mean.
9·0	*9·6	*8·0	*9·4	9·2	8·8	*9·4	8·8
			S.E. 0·11				

* Yields differ significantly from Control at P = ·05.

TABLE V.

Number of marketable heads and percentage whiptail.

Treatment.	CENTRE C.		CENTRE D.	
	Marketable heads, hundreds per acre.	% Whiptail.	Marketable heads, hundreds per acre.	% Whiptail.
Control	31	38	47	32
Sodium molybdate 2 lb...	48	17	73	13
Sodium molybdate 4 lb. ..	49	7	72	1
Ground Limestone ..	48	13	66	14
Mean	44	19	64	15
S.E.	2·91	2·34	3·5	2·17

At Centre A treatments significantly increasing yield are ground limestone, sodium molybdate 4 lb., and the molybdate spray. Gypsum decreased the yield.

Centre D produced nearly 50 per cent. more marketable heads than Centre C, but at each centre sodium molybdate at both levels and ground limestone at 3 tons per acre gave nearly equal numbers of marketable heads. These numbers were considerably higher than those on the control plots, being nearly 2,000 heads per acre more. The total number of plants in each plot remained about the same, and the increase in marketable heads was almost entirely due to the large reduction in numbers of whiptail plants. Examining the figures for percentage whiptail, it can be seen that though ground limestone at 3 tons per acre gave about the same percentage of whiptail plants as did sodium molybdate at 2 lb., the latter substance at 4 lb. reduced the percentage still further, even (at Centre D) to 1 per cent.

Symptoms and chemical data. The plots were scored at intervals for the incidence of whiptail. At Centres A and B the number of whiptail plants decreased as the season advanced, presumably because the plants were receiving an increasing supply of molybdenum. The reverse, however, occurred at Centres C and D where the incidence of whiptail increased to almost the end of January. Leaf samples were taken once from each Centre. The chemical data, together with the incidence of whiptail at the time of sampling, are shown in Table VI.

TABLE VI.

Molybdenum and manganese contents of broccoli leaves and incidence of whiptail.

Centre.	Treatment.	Molybdenum p.p.m.	Manganese p.p.m.	Incidence of Whiptail %
A. *sampled* 14.9.48	1. Ammonium molybdate 8 lb.	0·80	115	0
	2. Ammonium molybdate 4 lb.	0·80	108	0
	3. Ammonium molybdate spray	0·21	108	0
	4. Ground Limestone	0·34	60	0
	5. Gypsum	0·05	120	10
	6. Sand	0·20	107	2
	7. Control	0·20	137	1
B. *sampled* 14.9.48	1. Ammonium molybdate 8 lb.	5·70	118	0
	2. Ammonium molybdate 4 lb.	2·80	126	0
	3. Ammonium molybdate spray	1·30	123	0
	4. Ground limestone	1·50	74	0
	5. Gypsum	0·30	92	0
	6. Sand	0·90	160	0
	7. Control	1·00	128	0
C. *sampled* 17.1.50	1. Sodium molybdate 2 lb.	0·32	150	17
	2. Sodium molybdate 4 lb.	0·39	215	7
	3. Ground limestone	0·35	40	13
	4. Control	0·08	410	38
D. *sampled* 17.1.50	1. Control	0·08	235	32

At Centre A the molybdenum contents of plants on the gypsum plot were lower than with any other treatment and were at a level associated with deficiency (Table III).

At Centre B the levels of molybdenum were high even on the control (1·00 p.p.m.) and the absence of whiptail corroborated this. At Centres C and D the molybdenum values of plants on the control plots had fallen to 0·08 p.p.m. whilst with the other treatments there was a fourfold increase. It is interesting to note that even at this high level (0·35 p.p.m.) a few whiptail plants were still present but these, presumably, if they had been sampled separately, would have contained less molybdenum than this.

FIELD EXPERIMENT ON CAULIFLOWER.

One experiment was carried out during the summer of 1949 on cauliflower (var. Majestic) at a centre in the Gower, South Wales. The parent material consisted of an Old Red Sandstone drift and the soil was acid. Whiptail in cauliflower and broccoli had been reported on the farm during 1948.

Due to drought conditions, the plant population on the plot was considerably reduced and the yield results were not suitable for statistical analysis. However, the numbers of marketable heads are given in Table VII together with information about soil pH, molybdenum and manganese values.

TABLE VII.

Number of marketable cauliflower heads. Chemical data expressed on dry matter. Leaves sampled 3rd August, 1949.

Treatment.	Soil pH.	Marketable heads, hundreds per acre.	Mo p.p.m.	Mn p.p.m.	Whiptail %
Control	4·8	6	0·06	460	75
Ground limestone, 5 tons p.a., applied 21.4.49 ..	7·2	20	0·12	172	15
Gypsum, 5 tons p.a. applied 21.4.49	5·3	14	0·10	253	25
Ammonium molybdate 4 lb. p.a., applied 21.4.49 ..	5·1	20	0·12	298	15

It can be seen that the yield of marketable heads is low in relation to the number planted (7,000 per acre). However, the use of limestone or ammonium molybdate increased the yield threefold above the control. Even gypsum doubled the yield which represents an effect not found in other trials. The molybdenum content of the whiptail plants (0·06 p.p.m.) is at the same level as found in molybdenum-deficient broccoli (see Table III). The effect of raising the soil pH by limestone was to double the molybdenum content compared with the control whilst ammonium molybdate trebled the amount (0·22 p.p.m.). The concentration of manganese was high in the whiptail plants but no symptoms of toxicity were observed.

DISCUSSION.

These experiments carried out in the South of England and Wales establish the wide distribution of molybdenum deficiency in broccoli and cauliflowers grown on acid soils. The environment probably accounts for the degree of whiptail in any particular year and observations have shown that the occurrence of drought periods accentuates molybdenum deficiency if it is likely to occur. In this respect, it is interesting to note that the 1949-50 trials which were carried out or started in a dry summer gave better responses to applications of molybdenum than did experiments conducted during 1948-49.

There is no reason to believe that all acid soils will show whiptail. This was borne out at Centre B where, although the soil reaction was strongly acid, no whiptail developed. Even the application of gypsum at this centre failed to induce whiptail although it lowered the molybdenum content in the foliage. At Centre A, gypsum increased whiptail and decreased the molybdenum content. A similar result to this was reported from Australia by Waring *et al.* (1947) where sulphur was used to lower the soil pH.

The symptoms of whiptail, particularly the strap-like leaf, resemble those found in sand culture by Hewitt and Jones (1947) but only when the plant has reaçhed a certain stage in its development. The leaf symptoms of mottling, shrivelling and early senescence occurring during the first three weeks of growth which have been reported in sand culture were not observed in these experiments.

The data for the molybdenum levels in whiptail plants agree closely with the results of Hewitt and Jones (1948) in sand culture and from the evidence presented here, whiptail leaves, sampled midway between the inside and the outside foliage of the plant, have a molybdenum content ranging between 0·02 and 0·10 p.p.m. irrespective of the particular time of sampling (September to January). It is difficult to define any threshold figure above which the deficiency does not manifest itself since other factors are involved. The molybdenum contents of whiptail and healthy leaves taken from the same field have, however, always shown marked differences in level.

Wallace *et al.* (1945) have shown that manganese is an injurious factor on acid soils and as molybdenum deficiency also occurs on acid soils the problem of manganese toxicity may occur as a secondary complication. A whiptail plant invariably has a high manganese content (see Tables VI, VII) and if the concentration is sufficiently high, symptoms of manganese toxicity occur. In these experiments there was no evidence that the symptoms of whiptail were complicated by those of manganese toxicity.

The practical aspect of this work centres on the prevention and cure of whiptail in the field. Apart from the fact that molybdates will correct the deficiency, liming at appropriate rates to raise the pH of the soil has proved effective in these experiments and is the corrective treatment generally to be recommended to the grower or farmer.

In the event of whiptail developing in a field it is possible to remedy the shortage of molybdenum in the foliage by using molybdate sprays. Apart from the case cited here (Centre A) numerous other trials with broccoli have shown that the first spray must be applied not later than August and that the number of sprays required depends on the amount of the deficiency. Winter sprays are ineffective.

It appears from the data presented that the effect of lime is not due to molybdenum supplied as an impurity but to the action of the lime in increasing the availability of molybdenum in the soil, particularly at pH values above 6·5. At the same time liming is also instrumental in lowering the manganese content in the plant. Where whiptail occurs on neutral soils, liming would be expected to be ineffective and in such instances the use of sodium molybdate dressings at 2-4 lb. per acre is to be recommended.

SUMMARY.

(1) Molybdenum deficiency (whiptail) in field specimens of broccoli and cauliflower is described. The visual symptoms show a wide variation in type.

(2) All varieties of broccoli and cauliflower observed in the field during these trials (1947-50) appeared to be susceptible to whiptail.

(3) Diagnostic techniques, both visual and chemical, are discussed.

(4) The deficiency occurs on soils derived from differing parent materials, but so far has only been observed on acid soils.

(5) Whiptail plants contained 0·02 to 0·08 p.p.m. molybdenum, whereas healthy plants contained 0·12 to 6·00 p.p.m. molybdenum.

(6) In two experiments with broccoli, gypsum lowered the molybdenum content of the plants.

(7) Whiptail plants contained considerable amounts of manganese, but in these experiments there was no evidence of manganese toxicity.

(8) Whiptail was prevented by the use of molybdates at 2 to 4 lb. per acre, and by ground limestone at rates which raised the soil pH to neutrality.

(9) The practical correction for whiptail is usually liming, the effect of the treatment being to increase the availability of molybdenum in the soil.

(10) Where whiptail occurs on neutral soils, dressings of molybdates at 2 to 4 lb. per acre should be given.

ACKNOWLEDGMENTS.

This work was carried out under grants made by the Agricultural Research Council to whom grateful acknowledgment is made.

My thanks are especially due to Mr. J. Redmond for all the chemical determinations, Mr. G. H. Jones for the photographs, Mr. S. P. Craze for assistance with the Cornish experiments, Dr. J. E. Watkin with the Welsh centres and Dr. D. Boyd for statistical assistance.

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(*Received* 27/4/50.)

THE RELATION BETWEEN THE RATE OF RESPIRATION OF AN APPLE FRUIT AND ITS CONTENT OF PROTEIN

I. THE VALUE OF THIS RELATION IMMEDIATELY AFTER PICKING

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It has already been shown that during the growth of an apple fruit from the end of the period of active cell division until the onset of the respiration-climacteric, the rate of respiration per unit protein is approximately constant (Hulme, 1939). Further data which support this finding have since been obtained and are included in the present paper. Since, then, the rate of respiration per unit protein (the R/P ratio) appears to be constant for the major portion of the development of the fruit on the tree, it is of interest to examine this ratio in relation to season and cultural conditions within a variety, and to see whether it varies greatly from variety to variety. It is known, for example, that varieties vary considerably in their rates of respiration, dessert varieties tending to have a considerably greater rate than culinary varieties. Further, for a particular variety, those samples which have a high rate of respiration tend to be poor keepers.

From data accumulated at Ditton Laboratory over a period of years it has been possible to examine the R/P value of two varieties (Bramley's Seedling and Early Victoria) of apples throughout the growing season and also to compare the R/P value of a range of varieties at a point just prior to the onset of the respiration climacteric. The results of such a study are presented in the present paper.

EXPERIMENTAL.

Preparation of material for analysis and methods of analysis.

The method of sampling, preparation for analysis and subsequent analysis as well as the method used in the determination of the respiration-rate (at 12° C.) of the fruit have already been given (Griffiths, Potter and Hulme, 1950).

While it is a simple matter to determine the average protein content of a sample of apples and to obtain a value which may be regarded with reasonable certainty as representing the protein content of the fruit on the tree immediately before picking (Hulme, 1939), it is difficult to obtain a direct measurement of the rate of respiration which can be referred *exactly* to this protein-value. It is possible to use a calculated initial rate or the measured rate at some interval after picking. The factors involved in obtaining an estimate of this initial rate for young apples have already been discussed (Griffiths, Potter and Hulme, 1950) and the true initial values of R for several populations of Bramley's Seedling apples have been calculated (Griffiths, Potter and Hulme, 1950).

There is, however, a reasonable justification—and this point is discussed at length in the paper quoted—for using, in studies such as the present one, the respiration rate at 40 hours after picking as the " initial " rate for all but very immature fruit. It should be emphasized that the " true " initial rate (I.R.) and the rate observed at 40 hours (40R) of Bramley's Seedling apples (and, presumably this is approximately true for other varieties) approximate to one another from about 60 days from petal fall (about the end of July) until the onset of the respiration climacteric.

Details of the fruit used.

(1) *Bramley's Seedling apples.* The source of the fruit and the values of the protein-content (expressed as mg. protein N per apple) and rate of respiration (both " IR " and " 40R ", expressed as mg. CO_2 per apple per 100 hours) for the fruit developing on the tree in 1936, 1938, 1939 and 1945 (respiration rate only) have already been presented (Griffiths, Potter and Hulme, 1950). It should be noted that the 1945 data were obtained from trees different from those of the three earlier series. The results for this variety obtained at two stages of development (see Table I) in 1937 have also been published (Hulme, 1948).

In another experiment carried out with Bramley's Seedling apples in 1936, several similar trees on the same plot at East Malling Research Station were injected with aqueous solutions of various nutrients* as part of a study of the effect of such nutrients on the nitrogen-metabolism of the fruit.

Samples of fruit from these trees were gathered on 17th October and their rate of respiration and protein content determined using the usual technique. A description of this experiment has been given elsewhere (Hulme, 1936). The data relevant to the present purpose have been abstracted from the general data obtained and are presented in Table I (under Bramley's Seedling, 1936, Inj.). Details of the injection procedure are of no significance to the present investigation but the compounds injected are indicated in the table as follows: " control "—water only injected; " Sod. P. "—sodium phosphate; " U "—urea; " UP "—urea and phosphate; " UF "—urea + fructose; " UG "—urea + glucose. The trees used in this injection experiment were not the same as those used in the other experiments with this variety. The results given for protein nitrogen are the average obtained from the separate analysis of four to five samples of 25 apples each. Unlike all the other respiration data presented in this paper the respiration results for the injection series were not obtained from samples made up in exactly the same way as the ones used for chemical analysis. They represent the average respiration of two fruits, determined separately, taken from the population from which the " chemical " samples were prepared and having a weight approximately equal to the average weight of the fruits comprising these chemical samples. The " R " and " P " values in this series, therefore, do not correspond so exactly to one another as in the case of all the other data; the R/P values are, consequently, less reliable.

(2) *Early Victoria* apples. The protein-content and rate of respiration of this variety during development on the tree in 1939 have already been given (Griffiths, Potter and Hulme, 1950).

The results given in Table I for 1938 were obtained from two samples of 25 fruits each gathered just before the onset of the respiration climacteric.

* In collaboration with Dr. W. A. Roach, East Malling Research Station.

(3) *Other varieties.* The three sets of data given for Worcester Pearmain apples were obtained from samples of fruit from three sources (two in Kent and one in Cambridgeshire) gathered at maturity but still in a pre-climacteric state.

The fruit of the remaining varieties (Beauty of Bath, Grenadier, Lord Derby, Sturmer and Newton Wonder) was taken from trees at East Malling Research Station and was gathered at the dates indicated. All the fruit was in a pre-climacteric state. The results for all these varieties are the mean for two carefully prepared samples of twenty-five fruits each.

TABLE I.

The protein-nitrogen content and respiration rate of several varieties of apples.

Variety and Year.	Date of picking.	Days from petal fall.	Fresh weight per apple gm.	Protein –N per apple mg.	Respiration rate per apple. mg. CO_2 per 100 hours. IR	40R
Early Victoria, 1938	25th July	—	62·5	19·5	—	98·5
Early Victoria, 1939	13th June	22	7·2	8·2	—	39·6
	27th June	36	17·3	11·5	—	51·4
	11th July	50	33·0	15·2	—	70·8
	25th July	64	52·0	18·8	—	88·0
	3rd Aug.	73	65·2	20·3	—	106·0
	11th Aug.	81	74·7	21·9	—	121·3
	21st Aug.	91	85·7	22·4	—	125·1
	30th Aug.	100	99·4	25·0	—	162·5
	7th Sept.	108	109·0	26·4	—	178·2
	19th Sept.	120	119·5	29·3	—	224·8
	28th Sept.	129	124·3	31·9	—	244·9
Beauty of Bath, 1938	4th Aug.	—	72·2	32·1	—	234·6
Grenadier, 1938	1st Sept.	—	144·0	39·3	—	187·2
Lord Derby, 1938	5th Sept.	—	159·8	54·9	—	223·9
Sturmer, 1938	23rd Sept.	—	119·3	38·7	—	176·1
Newton Wonder, 1938 . .	26th Sept.	—	161·9	36·0	—	130·1
Worcester Pearmain, 1935 (1)	11th Sept.	—	88·0	27·7	—	123·0
(2)	11th Sept.	—	82·0	27·5	—	133·6
(3)	18th Sept.	—	81·8	28·2	—	124·3
Bramley's Seedling, 1936, Inj.						
Control	8th Oct.	—	110·7	31·2	—	93·0
Sod. P.	8th Oct.	—	115·7	31·3	—	86·0
U	8th Oct.	—	123·6	34·7	—	112·3
UP	8th Oct.	—	123·0	33·7	—	89·5
UF	8th Oct.	—	118·2	33·8	—	96·2
UG	8th Oct.	—	119·3	35·6	—	105·0
Bramley's Seedling 1945 . .	14th May	18	1·89	*5·07	54·9	17·9
	28th May	32	10·6	12·3	101·7	47·0
	5th June	40	15·6	14·7	94·8	56·6
	14th June	49	24·9	17·3	76·0	66·2

* Includes embryo seeds since these were too small to remove.

RESULTS.

The data for the protein-nitrogen content and rate of respiration not already presented elsewhere are shown in Table I. The results given in this Table, together with the published data referred to above, have been used to calculate the R/P values in Table II.

TABLE II.

The ratio : rate of respiration/protein content for several varieties of apples.

Variety and Year.	Days from petal fall.	IR/P.	40R/P.	Variety and Year.	Days from petal fall or date of picking.	40R/P.
Bramley's Seedling, 1936	21	6·4	3·9	Bramley's Seedling,		
(petal fall = 21st May)	33	7·3	3·7	1936, Inj. Control	8th Oct.	3·0
	46	6·3	3·9	Sod. P.	8th Oct.	2·8
	56	4·9	3·5	U	8th Oct.	3·2
	77	3·4	3·3	UP	8th Oct.	2·7
	94	—	3·3	UF	8th Oct.	2·9
	102	—	3·3	UG	8th Oct.	3·0
	110	—	3·2			
	123	—	3·3	Early Victoria, 1938	25th July	5·0
	137	—	3·4			
	145	—	3·5	Early Victoria, 1939	22	4·9
	159	—	3·7	(petal fall = 22nd May)	36	4·5
					50	4·7
Bramley's Seedling, 1937	31	—	4·5		64	4·7
(petal fall = 17th May)	78	—	4·6		73	5·2
					81	5·5
Bramley's Seedling, 1938	34	5·2	3·7		91	5·5
(petal fall = 12th May)	62	3·6	3·3		100	6·5
	95	—	3·6		108	6·8
	126	—	3·7		120	7·7
	146	—	4·2		129	7·9
	166	—	5·3			
Bramley's Seedling, 1939	24	8·2	3·8	Beauty of Bath, 1938	4th Aug.	7·3
(petal fall = 22nd May)	49	5·1	3·6			
	66	3·8	3·5	Grenadier, 1938	1st Sept.	4·8
	92	—	3·3			
	114	—	3·2	Lord Derby, 1938	5th Sept.	4·1
	135	—	3·4			
	157	—	4·8	Sturmer, 1938	23rd Sept.	4·6
	164	—	5·4			
				Newton Wonder, 1938	26th Sept.	3·6
Bramley's Seedling, 1945	18	10·8	3·5			
(petal fall = 26th April)	32	8·3	3·8	Worcester Pearmain		
	40	6·5	3·8	1935 (1)	11th Sept.	4·4
	49	4·4	3·8	(2)	11th Sept.	4·8
				(3)	18th Sept.	4·4

The values of the ratio IR/P and 40R/P were obtained by dividing the calculated initial rate of respiration (Griffiths, Potter and Hulme, 1950) and the observed "40 hour" rate of respiration (at 12° C.) in mg. CO_2 per apple per 100 hours respectively by the corresponding protein-N content in mg. per apple. It has been impossible to calculate the true initial rate of respiration for varieties other than the Bramley's Seedling since data relating IR and 40R are only available for this variety and it cannot be assumed that the relationship between the two rates is the same for all varieties of apple.

DISCUSSION

It has already been shown (Griffiths, Potter and Hulme, 1950—see also Kidd and West, 1945) that the respiration rate of Bramley's Seedling (and Early Victoria) apples in the early stages of development falls rapidly after the apples have been picked. As the fruit develops the speed and extent of this fall in respiration decreases. At the stage of maturity reached at 50-60 days from petal fall there is relatively little change in respiration rate during the first 40 hours after picking. Other data, as yet unpublished, have shown that while the protein-content of these young fruits also falls steadily after picking, the fall does not become appreciable for several days after picking and the overall rate of fall is less than the rate of fall of the respiration. This course of events is reflected in the rapid decrease in the ratio IR/P for Bramley's Seedlings up to 50-60 days from petal fall shown in Table II. It might be postulated that this high rate of respiration per unit protein—high, that is, in relation to the period of growth between 50-60 days from petal fall and the onset of the respiration climacteric—is a result of the intense cellular activity associated with rapid cell division. This hypothesis cannot, however, be maintained, for Smith (1950) has shown that in the fruits of both Early Victoria and Bramley's Seedling apples, active cell division ceases about the end of June (30-35 days from petal fall). Until more data are available this problem cannot usefully be pursued further. Possible factors involved are the level of the nutrient (carbohydrate, nitrogenous compounds etc.) supply entering the fruit and the pH and rH of the tissue. The relative ease with which young fruits darken on cutting is evidence of a high oxidation reduction potential in these tissues.

The fact remains, however, that from about the end of the period of cell division until the onset of the respiration climacteric (about 90 days and 125 days from petal fall for Early Victoria and Bramley's Seedling apples respectively) the ratio 40R/P remains remarkably constant. As mentioned earlier, IR and 40R differ very little after approximately 60 days from petal fall.

The value of 40R/P at any time from about 60 days after petal fall until the onset of the respiration climacteric may, therefore, be reasonably taken as a physiological constant for a set of trees of the same variety for a given season (Table II). An examination of the values of 40R/P (hereafter written simply as R/P) for Bramley's Seedling and Early Victoria apples given in Table II suggests that this ratio is not only constant for the same trees in the same season but also for the same trees in different seasons. For example the average values of the ratio (between approximately 60 days and 120 days from petal fall) for Bramley's Seedlings from the same trees in 1936, 1937, 1938 and 1939 is 3·3, 4·6, 3·5 and 3·4 respectively. The apparent anomaly in 1937 may be explained by the fact that 1937 was a season of frost injury and the crop was a small one of rapidly growing fruit—the average weight per apple at about 80 days from petal fall was 102·5 gm. (Hulme, 1948, p. 343) whereas the average weight at about the same time in the case of the other three seasons was 57 gm., 79 gm. and 74 gm. respectively (from Griffiths, Potter and Hulme (1950) and Table I, above).

If we now consider the value of R/P of fruit of the same variety but from different sources, it is clear that here again the ratio is reasonably constant. For Bramley's Seedling apples, 1945—these were younger trees grown under poorer general cultural conditions—the value was 3·8. Again, in the injection experiments of 1936, in spite of an appreciable effect of the various injections on the rate of respiration (Hulme,

1936) the value of R/P only varied between 2·7 and 3·2. The general high level of nutrition in all these trees may be correlated with the rather low value of R/P. The respiration rate per unit protein of mature, pre-climacteric Early Victoria apples from one source in 1938 was 5·0, and from another source in 1939 the average value, between 60 days from petal fall and the onset of the respiration climacteric, was 5·2. For Worcester Pearmain from three different sources in 1938 the ratio was 4·4, 4·8 and 4·4.

The question of the value of the R/P ratio in relation to varietal differences may now be raised. In the range of culinary varieties given in Table II, the slow maturing varieties Bramley's Seedling and Newton Wonder exhibit a relatively low value for the ratio—the average for all the Bramley's Seedling samples (excluding 1937) was 3·4 and for Newton Wonder, 3·6. The early maturing Early Victoria gave the highest value, 5·1 and the " intermediate " varieties Grenadier and Lord Derby gave values of 4·8 and 4·1 respectively.

Unfortunately results are available for only three dessert varieties, the early maturing Beauty of Bath, the later Worcester Pearmain and the late Sturmer. The range here is 7·3, 4·6, 4·6. Although no satisfactory data are available for Cox's Orange Pippin, indications are that the R/P value is at least as high as that for Beauty of Bath apples.

On the data presented it would appear, therefore, that the rate of respiration per unit protein (R/P value) is constant in pre-climacteric apples at least subsequent to 60 days from petal fall and that this constant is characteristic, within fairly narrow limits, of a variety. Dessert varieties exhibit a higher rate of respiration per unit protein than culinary ones. It remains to be seen what relation this constant bears to widely different cultural conditions and to storage behaviour over a wide range of apple varieties.

SUMMARY.

(1) Data are presented which show that, subsequent to about 60 days from petal fall until the onset of the respiration climacteric, the rate of respiration of an apple on the tree is proportional to its content of protein. With less developed apples the ratio rate of respiration to protein content (R/P ratio) appears to be falling from a relatively high value a few days after petal fall.

(2) In mature, pre-climacteric apples the R/P ratio is shown to be approximately constant for a given variety, at least when cultural conditions are not very different.

(3) It is suggested that the R/P ratio has a value characteristic of a variety and that the value tends to be higher for dessert varieties of apples than for culinary ones.

ACKNOWLEDGMENTS.

Much of the experimental work was done by Mr. W. Arthington.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research, and is published by permission of the Director.

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(*Received* 6/5/50.)

THE USE OF *ASPERGILLUS NIGER* (M)* FOR THE DETERMINATION OF MAGNESIUM, ZINC, COPPER AND MOLYBDENUM AVAILABLE IN SOILS TO CROP PLANTS

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SYNOPSIS

The fungus *Aspergillus niger* (Mulder's M strain) requires the following mineral elements for normal growth ; nitrogen, phosphorus, potassium, magnesium, sulphur, iron, zinc, copper, manganese, molybdenum, and possibly gallium and vanadium. When one of the essential mineral elements is omitted from the culture solution, the growth of the fungus is markedly reduced, and the addition of increasing amounts of the element, from deficiency to sufficiency levels, results in an increase in dry-weight yields and sporulation. The unpurified mineral macronutrients and dextrose that ordinarily comprise culture solutions contain sufficient of the micronutrient elements to provide for the normal growth of the fungus. Therefore chemical and ion-exchange methods are used to remove the micronutrient to be determined from the mineral macronutrient and dextrose so that a standard growth series can be prepared for the " test " element, from deficiency to normal levels. A determination of the " test " nutrient is made by adding a known amount of material, e.g. soil, extracts of fresh plant tissues, to 50 ml. of culture solution containing all the mineral elements other than the one to be determined. By reference to a standard growth series, using as criteria for assessment the development of spores and dry-weight yields of the fungus, the amount of the element present in the test substance is determined.

The method has been used to determine normal and deficiency levels of magnesium, copper, zinc and molybdenum in British soils. The bioassay method for molybdenum is very sensitive, and can be used to determine levels of the element in tissue extracts and in the ash of plants which are not detected by chemical methods.

MICROBIOLOGICAL methods for the determination of mineral nutrients in soils using fungi of the genus *Aspergillus* were introduced by Butkevič (1909) and Kosceleckiĭ (1909) for the study of available phosphorus in Russian soils. Benecke and Söding (1927), Niklas *et al.* (1930), and Gerretsen (1948), have also used the method for the same purpose, whereas Mehlich *et al.* (1933) and Sartory *et al.* (1935) used it to determine available potassium in soils. Smith (1934, 1936) and Goodwin (unpublished data) have determined both mineral nutrients in British soils using the fungus. Other fungi used in similar soil studies include *Cunninghamella* sp. by Mehlich (1934) and *Rhizopus* sp. by Seidel (1931).

Bioassay of mineral nutrients in soils using bacteria has been attempted by Conn (1928), Conn and Darrow (1938), Taylor (1938), using *Bacterium globiforme*, and by Christensen (1906) using *Azotobacter* sp. It appears that neither the presence of these organisms nor their numbers in soil is a reliable index of the K, P and Ca status of the soil. *Lactobacillus arabinosus* (Bentley *et al.*, 1947) and *Streptococcus faecalis* (Barton Wright, 1945) have been suggested as indicators of Mn and K levels respectively. Morgan (1933) has used algae as qualitative indicators of soil fertility.

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* *Aspergillus niger* (Mulder strain) kindly supplied by Dr. E. G. Mulder, Groningen, Holland, was used in this investigation.

The importance of mineral micronutrients for the metabolism of *Aspergillus niger* was first shown by Raulin (1869) who demonstrated the essentiality of Fe and Zn for optimum growth. The use of pure nutrients in culture work by Bertrand and Javillier (1911), Roberg (1928), Metz (1930), Steinberg (1935), Sakamura (1936) and Gollmick (1936) showed that Fe, Zn, Cu, Mn, Mo and possibly Ga are essential micronutrients. Steinberg (1936) has suggested that optimum levels for these are: iron 0·2, zinc 0·18, copper 0·04, manganese 0·02, molybdenum 0·02 and gallium 0·02 mg. per litre. Bertrand (1941) has observed that the addition of vanadium to a culture solution containing the above micronutrients may result in increased growth of the fungus. Thus, as a result of pure culture work, the Arndt-Shultz law of chemical stimulation of organisms by heavy metals resulting in abnormal growth and applied to micronutrient effects on plant growth by Pfeffer (1895) was replaced by the concept of the essential nature of the mineral micronutrients for optimum growth.

In recent years, bioassays have been made of the micronutrients in soils by the use of *Aspergillus niger*. Thus, Mulder (1939) after a comprehensive study of a number of strains of *Aspergillus niger*, chose one, designated M strain, for the bioassay of Cu in Dutch soils with special reference to Cu deficiency, " Ontginningsziekte " or reclamation disease. Moreover with Smit (1940) he studied available Mg in soils where " Hooghalen " disease occurred in cereals. Acock (1941) used the Neuberg ex Lister 2380 strain of the fungus, to examine suspected Cu deficiency in Australian soils from areas where " coast disease " and " steely wool " in sheep and " withertip " symptoms in cereals, are common. In this country preliminary work on the use of the fungus for the bioassay of Mg, Cu, Zn and Mo in soils has been reported (Nicholas and Fielding, 1947, Nicholas, 1949, Nicholas and Fielding, 1950).

This paper deals with the bioassay of Mg, Cu, Zn and Mo in soils using *Aspergillus niger* (Mulder's M strain) and the preparation of culture solutions free from Mg, Fe, Cu, Zn, Mn and Ga respectively. Using this method, it has been possible to demonstrate deficiency levels of magnesium, copper, zinc and molybdenum respectively in British soils.

Basis of bioassay method.

The method of bioassay is based on the fact that the fungus will not complete its life cycle unless certain mineral nutrients are present in the culture solution, viz. the macronutrients, nitrogen, phosphorus, potassium, magnesium and sulphur, and micronutrients, zinc, copper, iron, manganese, molybdenum, and possibly gallium and vanadium. Moreover the response to the addition of an essential mineral element, from deficiency to sufficiency levels, when others are present in adequate supply, is specific and quantitative. Standard growth series for the different elements may be made either for use in making visual comparisons taking into account the growth of mycelium, development and colour of spores or for quantitative determinations of weights of mycelium. Thus for bioassay work, a known amount of the material for test is added to a culture solution containing all the mineral elements other than the one to be determined. The growth of the fungus under these conditions thus depends on the amount of the mineral nutrient to be tested that it derives from the added material which can be ascertained by reference to the growth standards.

EXPERIMENTAL.

CULTURE SOLUTIONS.

Composition.

The culture solutions used (A and B) are substantially the same as those recommended by Mulder (1939-1948), having the following composition :

A

Dextrose	50 gm.	$FeCl_3 \cdot 6H_2O$	20 mg.
KNO_3	5 gm.	$ZnSO_4 \cdot 7H_2O$	10 mg.
K_2HPO_4	2·5 gm.	$MnSO_4 \cdot 4H_2O$	3 mg.
$MgSO_4 \cdot 7H_2O$	1 gm.	$Na_2MoO_4 \cdot 2H_2O$	1·5 mg.
$Na_2SO_4 \cdot 10H_2O$	1 gm.	$CuSO_4 \cdot 5H_2O$	1 mg.

Glass-distilled water 1 litre.

B

Dextrose	50 gm.	$FeCl_3 \cdot 6H_2O$	3 mg.
KNO_3	5 gm.	$ZnSO_4 \cdot 7H_2O$	2 mg.
KH_2PO_4	0·6 gm.	$MnSO_4 \cdot 4H_2O$	0·3 mg.
$MgSO_4 \cdot 7H_2O$	0·6 gm.	$CuSO_4 \cdot 5H_2O$	0·6 mg.
$CaSO_4 \cdot 2H_2O$	0·5 gm.		

In addition, culture solution (C) is used at Long Ashton.

C

Dextrose	50 gm.	$FeCl_3 \cdot 6H_2O$	3 mg.
KNO_3	5 gm.	$ZnSO_4 \cdot 7H_2O$	2 mg.
NaH_2PO_4	0·6 gm.	$MnSO_4 \cdot 4H_2O$	0·3 mg.
$MgSO_4$	1 gm.	$Na_2MoO_4 \cdot 2H_2O$	0·5 mg.
$Ca(NO_3)_2$	0·6 gm.	$CuSO_4 \cdot 5H_2O$	0·6 mg.

$Ga(NO_3)_2 \cdot 8H_2O$	0·2 mg.
$Co(NO_3)_2 \cdot 6H_2O$	0·1 mg.
$Ni(NO_3)_2 \cdot 2H_2O$	0·08 mg.
$NaVO_3 \cdot 4H_2O$	0·08 mg.

The nutrients, which must be the A.R. or C.P. grade, are dissolved in one litre of glass-distilled water and 50 ml. is used for each culture. When purification of the culture solution is necessary the macronutrients and dextrose are made up without addition of the micronutrients and, after elimination of the element to be tested the remaining trace element supplement is added.

Culture solution A may be used for the bioassay of Cu or Mg and for this purpose $CuSO_4$ and $MgSO_4$ respectively are omitted. Basal solution B was used by Mulder (1948) for the bioassay of Mo. Solution C has been developed at Long Ashton, and its main advantages over the others are that $Ca(NO_3)_2$ is more soluble than the sulphate and the elements of Co, Ni, V and Ga are added after the purification processes in case of partial removal of these either by chemical co-precipitation or ion exchange methods.

Purification.

In order to study the effects of specific mineral nutrients, in particular micronutrients, on the physiology of *Aspergillus niger* (M) it is necessary to use chemical or ion exchange methods to remove them from the culture solution. Most A.R. chemicals contain sufficient quantities of micronutrient elements to provide for the growth requirements of the organism, and for Fe, Zn, Cu, Mn, Mo and Ga, it is necessary to eliminate them in turn from the culture solution to prove that they are essential micronutrients. Methods used for the removal of a particular nutrient should not affect other constituents of the media. A number of methods have been proposed for the purification of culture solutions. Steinberg (1935) used $CaCO_3$ to co-precipitate undesirable heavy metals as phosphates, hydroxides, carbonates and basic carbonates, and Stout and Arnon (1939) have used a modified method for the removal of Zn, Cu and Mn. Sakamura (1936) employed washed $Ca_3(PO_4)_2$ as an adsorbing agent in acid solution at pH 5·5 whereas Roberg (1928), Bortels (1930), Mulder (1939) used activated charcoal in the presence of a sulphide. Bertrand (1942) removed Mn by oxidizing the ion to MnO_2 followed by its adsorption on $Mg(NH_4)PO_4$.

TABLE I.

Purification of macronutrients and dextrose.

Nutrient element removed.	Method used for its removal.
Magnesium . .	Recrystallization of macronutrients or precipitation with 8-hydroxyquinoline at pH 8, preferably in presence of added $MgSO_4$ (in absence of phosphates). Remove excess quinoline with redistilled ether.
Copper	Elution with dithizone in CCl_4 at pH 4 or co-precipitation with Cu or Cd as a sulphide using H_2S, and elimination of latter by boiling.
Zinc	Elution with dithizone in CCl_4 at pH 6 or co-precipitation with Fe using 8-hydroxyquinoline at pH 5. Addition of redistilled ether to remove excess quinoline. Basal solution may be eluted with 8-hydroxyquinoline and then extracted with redistilled chloroform and ether.
Iron	Co-precipitation in the presence of added Al and Fe, using 8-hydroxyquinoline at pH 5 or addition of cupferron; removal of iron cupferrate with redistilled ether, addition of Cu to remove excess cupferron and H_2S precipitation to remove excess Cu; eluting basal solution with 8-hydroxyquinoline as for Zn.
Molybdenum . .	Co-precipitation with Fe and Al using 8-hydroxyquinoline or preferably as sulphide in presence of Cd, Cu, Hg and Pb using H_2S. The addition of I_2 in KI simultaneously with H_2S precipitation facilitates further removal of Mo.
Manganese . .	Extraction with sodium diethyldithiocarbamate in CCl_4 and removal of excess of organic reagent with Cu; precipitation of Cu as sulphide with H_2S.
Gallium	Co-precipitation with copper as cupferrates using cupferron. Removal of excess metal cupferrates with redistilled ether and excess Cu as sulphide using H_2S; or use of 8-hydroxyquinoline at pH 6 to form gallium quinolate in presence of added Ga (5 μg), and extraction of the complex in chloroform. The extraction can be checked as gallium quinolate is fluorescent under U.V. illumination.

Among chemical methods recently described is that in which 8-hydroxyquinoline and similar compounds are used to form complexes with metallic ions (Waring and Werkman, 1943, Albert and Gledhill, 1947, Albert and McGrath, 1947). The complex as well as the residual reagent is usually removed by chloroform and ether extraction. Other complex-forming reagents used are ferro and ferricyanide (Mezzadroli, 1938), calcium sulphide (Boehm, 1947), hydroxy-organic acids (Hutner, 1946), peroxides (Lorenz, 1940). The use of cation-exchange materials has been suggested for the purification of dextrose in the media, but difficulties have been encountered with special culture solutions containing other organic constituents (Perlman, 1949). Electrolytic deposition of Cu was proposed by Wolff and Emmerie (1930), and Molisch (1892), Roberg (1928) and Molliard (1929) have used *Aspergillus niger* to remove the iron from culture solutions to be used as a basal medium for iron deficiency studies.

CHEMICAL METHODS.

The chemical methods for the purification of the salts of the macronutrient elements and of dextrose used at Long Ashton are summarized in Table I. The micronutrients Fe, Zn, Cu, Mn, Mo, Ga, Co and Ni, with the exception of the one to be determined, are added later to the purified media. They must each be of A.R. grade and it is advantageous to check spectrographically their purity before use where this is possible.

The following notes give the procedures followed for tests for Mg, Cu, Zn and Mo :

Magnesium.

Culture solution A or C is used for the bioassay of magnesium ; the $MgSO_4$ is omitted. It may be necessary to remove Mg from the mineral macronutrients and this is done either by recrystallization of individual salts and enhancing yields with redistilled alcohol or by passage through a cation exchange resin. Although this method of removal is adequate to inhibit growth of *Aspergillus niger*, it may not be sufficiently refined for bacterial work. In this instance 8-hydroxyquinoline is used to remove Mg in alkaline solution as follows : to a solution of the mineral macronutrients (in the absence of phosphates) including dextrose, 5 ml. of 2 per cent. 8-hydroxyquinoline in 2N acetic acid is added and then sufficient ammonia (pure) to adjust the pH to 8. The solution is placed in an incubator for twenty-four hours, at a temperature of 33° C. The precipitate is filtered and excess quinoline is removed by eluting the solution several times with 50 ml. portions of redistilled chloroform and then with redistilled ether to ensure the complete removal of toxic products. The pH is adjusted to 5 with HCl (redistilled) and micronutrients viz. Fe, Zn, Cu, Mn, Mo and phosphate (previously recrystallized) are added and the solution made up to a litre. It has been observed that the addition of magnesium sulphate to a solution of the macronutrients before purification facilitates the removal of the last traces present as an impurity. 50 ml. portions of the culture solution are transferred to one litre pyrex conical flasks. The standard solutions of Mg used are as follows : 0, 25, 50. . . .500 μg Mg respectively in steps of 25 μg. To prevent contamination of the standards 100 ml. pyrex beakers are placed over the necks of the flasks and the series autoclaved at 120° C. and 15 lb. pressure per square inch for 15 minutes. The solutions are then cooled and inoculated with four drops of a spore suspension of the fungus and placed in the incubator at 25° C. for six days.

Micronutrients : Cu, Zn, and Mo.

The bioassay of micronutrients involves the purification of distilled water, inorganic salts, and dextrose (Table I) of the culture solution. Distilled water from a tinned copper still may contain as much as 10 μg Cu per litre or 0·5 μg per 50 ml. of culture solution, which is sufficient to support a mycelium covered with light brown spores. Double distillation of this water in a pyrex apparatus effectively reduced the Cu level to $< 0{\cdot}1$ μg per litre or 0·005 μg per 50 ml. culture solution. Pyrex conical flasks (1 litre) or petri dishes (6 in. diameter) must be used throughout to minimize the risk of contamination. Glassware must be thoroughly cleaned with hot redistilled HCl and rinsed finally with glass-distilled water.

The inorganic salts comprising the major constituents, viz. KNO_3, KH_2PO_4 etc. and dextrose were initially purified by a modification of the adsorption methods used by Steinberg (1935) and Stout and Arnon (1939). The modified method involves the steaming of the macronutrients and dextrose in 500 ml. of distilled water in the presence of 10 gm. $CaCO_3$, 10 ml. 20 per cent. $CaCl_2$ and K_2HPO_4 respectively and 10 gm. of NaHCO for 15 minutes at 100° C. This was found to be reasonably effective in eliminating Cu, Zn, Fe and Mn but had no effect on Mo. When this procedure was used, however, it was not always possible to reproduce a standard growth series, presumably due to the adsorption of the added micronutrients by excess calcium phosphate remaining in solution. Details of procedures adopted for the preparation of standard growth curves for Cu, Zn, and Mo are as follows :

Copper. The macronutrients and dextrose are dissolved in 200 ml. of glass-distilled water and the solution is transferred into a 500 ml. pyrex separating funnel. 10 ml. portions of 0·02 per cent. dithizone in CCl_4 are added and the extraction continued until no more Cu can be detected as a red dithizonate. The solution is then eluted with redistilled CCl_4 to remove any residual dithizone. The micronutrients Fe, Zn, Mn, Mo, Ga, V, Ni and Co, at the appropriate levels, contained in 1 ml. of glass-distilled water respectively, are then added and the solution made up to a litre with glass-distilled water.

Another effective method is to add 2 ml. 5 per cent. $CdCl_4$ or $CuSO_4$ to a solution of the inorganic macronutrients and dextrose, and co-precipitate CuS with CdS by passing H_2S through the solution for five minutes. The sulphide is allowed to settle as a precipitate and after two hours the solution is filtered through a Whatman No. 42 paper. Excess H_2S is eliminated by boiling. The micronutrients other than copper are added and the solution made up to a litre with glass distilled water. This method is probably simpler than the former and is usually more effective.

50 ml. portions are transferred into conical flasks or Petri dishes and the following standard series is easily reproduced : 0, 0·05, 0·1, 0·2, 0·4, 0·6, 0·8, 1, 1·5, 2, 3μg Cu per 50 ml. culture solution. Incubation takes place at 25° or 31° C for either six or five days respectively.

Zinc. A solution of the macronutrients and dextrose is buffered to pH 5·5 to 6 and eluted with dithizone as described for Cu. At this pH Cu and Zn are removed. After elution with redistilled CCl_4 the micronutrients are then added and the solution made up to 1 litre.

Zn is precipitated in the presence of Fe with 8-hydroxyquinoline as follows : to a solution of macronutrients at pH 5·5 are added 1 ml. $FeCl_3$ (20 mg. Fe per ml.) and 5 ml. of 2 per cent. 8-hydroxyquinoline in 2N acetic acid and the whole is allowed to stand at 33° C. for 24 hours. The precipitate is filtered off through acid-washed filter paper and excess quinoline removed with redistilled chloroform and ether respectively. Several elutions may be necessary to rid the solution of quinoline. After addition of micronutrients, other than Zn, the solution is made up to a litre.

50 ml. portions are taken in either conical flasks or Petri dishes and the following standard series used: 0, 0·25, 0·5, 0·75, 1, 2, 3, 4, 5 and 10 μg Zn respectively.

Molybdenum. The molybdenum can be co-precipitated in the presence of Fe or Al at pH 5 with 8-hydroxyquinoline as described for Zn.

The co-precipitation of molybdenum sulphide with CdS, CuS, HgS, and PbS is very effective in eliminating the last traces of Mo. Pb is included to remove molybdate which may be an impurity in the macronutrients. The addition of 2 ml. of 1 per cent. I_2 in 4 per cent. KI simultaneously with H_2S precipitation of the metals facilitates further removal of Mo. This is presumably due to the formation of colloidal S which acts as an additional collector. The standard series is as follows: 0·5, 1, 5, 10, 25, 50, 75, 100, 200, 500 $\mu g \times 10^{-4}$ Mo in 50 ml. culture solution respectively.

ION EXCHANGE METHODS.

Ion exchange methods have also been used to purify the culture solutions from micronutrients. Cation exchangers (A.R. grade) viz. amberlite IR 100, IRC 50 and Zeocarb 215 respectively may be used for this purpose. It is necessary to remove traces of micronutrients from the resins before use. Iron and copper appear to be the main contaminants. The resins are prepared as follows: 50 gm. of resin is placed in a pyrex glass column and a 5 per cent. solution of $MgSO_4$ is percolated through continuously to remove iron and copper. When the two micronutrients cannot be detected chemically in the eluant, 2N HCl is used to remove Mg and a continuous percolation is maintained until Mg is not detected in the extract. The resin is then washed in the percolating apparatus with glass-distilled water until the pH of the effluent is constant.

The non-ionic component of the culture solution, viz. dextrose, can be purified by passage through the cation exchanger. Thus 50 gm. of dextrose dissolved in 100 ml. distilled water is passed several times through columns of the resin, viz. 10 gm. of resin contained in pyrex tubes fitted with glass wool plugs at the base, as a support. In this way pure dextrose solution is prepared free from micronutrients.

An attempt has been made to purify the mineral macronutrients as well as dextrose by ion exchange methods. It is found that about half of the macronutrients in the basal culture solution are removed after passage through the resins. The macronutrient salts of the basal media contained in about 100 ml. of glass-distilled water were passed several times through cation exchangers Amberlite IR100 or IRC 50 or Zeocarb 215. Other batches were also passed through anion exchange columns IR4B after cation exchange treatment. Bioassay tests for the micronutrients in the final eluant are expressed as μg per 50 ml. of the basal culture solution. Comparative results obtained by the ion exchange methods and by chemical methods of purification are given in Table II.

Zeocarb 215 or Amberlite IR100 are equally effective in removing the micronutrients but Amberlite IRC50 is a weak exchanger. The passage of the solutions through anion columns after cation exchange removed further amounts of Zn, Fe and Mo. The reason for further removal of Zn and Fe is not clear, but it may be the result of their precipitation as phosphates and hydroxides under the alkaline conditions of anion exchange. The phosphate in the basal culture solution is markedly

TABLE II.

Comparison between ion-exchange and chemical methods for the removal of mineral micronutrients from mineral macronutrients and dextrose.

Element.	Cation exchange method.			Cation and anion exchange method.	Chemical methods as in Table I.
	Zeocarb 215.	Amberlite.		Zeocarb 215 or Amberlite IR100 treatment followed by passage through Amberlite IR4B.	
		IR100.	IRC50.		
Cu as μg/50 ml. culture solution	0·2	0·1	2	0·1	0·05
Zn as μg/50 ml. culture solution	1	1	5	0·1	0·1
Fe as μg/50 ml. culture solution	0·5	0·5	5	0·1	0·1
Mn as μg/50 ml. culture solution	0·01	0·01	10	0·01	0·01
Mo as μg/50 ml. culture solution	0·01	0·01	>0·5	0·001	0·00005

reduced after passage through the anion column. The further removal of molybdenum after anion exchange may be due to its removal as molybdate. This is confirmed by the chemical method of purification, as the addition of Pb to form lead molybdate improves the efficiency of the removal of molybdenum.

The results of ion exchange methods for the removal of micronutrients from the macronutrient salts and dextrose compare favourably with those of chemical procedures. The main disadvantage is the non-selective removal of mineral micronutrients and macronutrients. Thus after ion exchange the macronutrients in the basal culture solution are not only markedly changed, but the treatment may result in a deficiency of a macronutrient. Further work is in progress to overcome this difficulty. Ion exchange methods are still in the experimental stage and cannot as yet be recommended for routine use. Chemical purification methods are more specific and do not affect the macronutrients of the basal solution.

PREPARATION OF SPORE SUSPENSIONS AND INOCULATION.

Aspergillus niger (M) is subcultured on nutrient agar slopes at 25° C. for six days so as to produce a dense black spore cover. The inoculation of fresh agar slopes is made from the original culture so that the risk of mutation is minimized. The agar slope is thoroughly combed with a sterile platinum wire and the spores deposited in a pyrex test tube containing about 10 ml. of glass-distilled water. In this way a thick suspension of spores is made for inoculation of the cultures.

Four drops of spore suspension are introduced into 50 ml. of the sterile culture solution. When conical flasks are used, pyrex beakers are inverted over the neck instead of using cotton wool neck plugs. This reduces the risk of metal contamination.

Incubation for five days at 31° C. or six days at 25° C. gives satisfactory results.

TABLE III.

The growth of Aspergillus niger in relation to magnesium content of culture solution. (Grown at 25° C. for six days.)

Mg. as μg. in 50 ml. culture solution.	Description of mycelium and spore cover.	Dry weights of mycelium in mg.	pH of culture solution after incubation.	Designation in Fig. 1.
0	No appreciable growth	0 to 23	3·87	a
25	Thin wefts of mycelium	47·5	4·82	b
50	Incomplete mycelium with few underdeveloped pale brown spores	123·1	3·63	c
75	Complete cover of mycelium with few scattered pale grey brown spores	332·2	3·28	
100	Thicker mycelium with a greater number of grey brown spores	407·8	3·34	d
150	Abundant grey brown spores	554·6	3·17	e
200	Completely formed undulating mycelium with grey black spores	610·4	2·06	f
250	Black spores dispersed between grey black spores	647·8	2·18	
400	Surface of mycelium nearly covered with black spores	684	3·11	h
500	Complete black spore cover	779	2·92	i
1,000	Complete black spore cover	895	2·41	

SOIL SAMPLING AND PREPARATION OF SOIL FOR BIOASSAY.

Soil samples for micronutrient assay must be taken adjacent to the growing plants and preferably from the rooting zone. Samples are taken with a soil auger or by removing normal and affected plants respectively (e.g. cereals and vegetable crops) and sampling the soil adhering to the roots. By sampling in this way differences in micronutrient status between normal and deficient soils are likely to be more pronounced than those for general soil samples.

Soils to be tested must be thoroughly dried in air before use and preferably passed through special nickel-plated sieves, 1 mm. mesh, and stored in air-tight, inert containers.

RESULTS.

ASSESSMENT OF RESULTS.

The criteria used to determine the amount of "test" nutrient are, intensity of sporulation, colour of spores and dry weight yields of the fungus. The elimination of Mg, Zn and Mo respectively from culture solutions results in a thin mucous mycelium devoid of spores, with a dry weight of approximately 50 mg. With increasing amounts of the " test " nutrient, when others are present in adequate supply, the mycelium thickens, spores appear, and the dry weight yields of the fungus increase to a maximum of approximately 950 mg. Dry weights of the fungus may increase with increasing amounts of the " test " nutrient even after full sporulation has been achieved and vice versa. Thus for assay of the " test " nutrient it is important to use the portion of the growth curve where intensity of spore cover and dry weights parallel one another. Copper has a specific effect on the pigmentation of the spores and colour changes from pale yellow to black spores between 0·05 and 1 μg. per 50 ml. culture solution, facilitate the determination of minute amounts of the element.

Magnesium.

Standard series. The growth and spore characters of *Aspergillus niger* in relation to added Mg at 0, 25, 50, ...1,000 μg are shown in Table III and Fig. 1. which show that differences in growth of mycelium, spore cover and dry weights are correlated with increasing amounts of magnesium.

Bioassay of soil. One gram portions of soil were used for the bioassay of Mg. Pre-treatment of soil was necessary in some instances to overcome interference from other fungi and bacteria which grew despite the fact that the culture solution was acid (pH 4). Treatments tried included preheating soil in an oven, autoclaving soil with the culture solution, and alcohol treatment (Acock, 1941) respectively.

The soils tested were taken from a long-term manurial trial at Long Ashton in which the following treatments are represented in three blocks, NPK, PK (omit N) and NP (omit K). In addition, magnesium sulphate at 1 cwt. per acre has been applied to block II annually since 1940 to offset symptoms of Mg deficiency observed in susceptible crops, viz. potato and cauliflower, especially in the PK (omit N) and to a lesser extent in the NPK treatments. The results of the bioassay method, made in triplicate, based on sporulation and dry weights of mycelium, are given in Table IV as well as results for other diagnostic methods.

The data in Table IV show that the Mg treatment given to block II is reflected by the results of the bioassay method and that similar values are obtained whether an autoclaved or heating pre-treatment is given to the soil. The exchangeable Mg, extracted with neutral ammonium acetate (Peach, 1941), shows similar trends to those of the bioassay results.

In Fig. 2 are shown the results of the bioassay of soil from the NP (omit K) and NPK treatments (blocks I or III) and NP (omit K) with magnesium sulphate (block II). Thus, using the bioassay method it is possible to detect a deficiency of Mg in the presence of a K shortage, a point which is not shown by the chemical analysis of potato foliage due to the interaction of the two nutrients within the plant (Table IV).

Copper.

Standard series. The standard growth series for copper is 0, 0·05, 0·1, 0·2, 0·4, 0·6, 0·8, 1·0, 2, 3, 5 μg Cu per 50 ml. culture solution as shown in Table V and Fig. 3.

The data show that with increasing amounts of Cu the formation of spores is increased and the colour of the spores also appears to be characteristic for each level of copper. The colour change facilitates the assay of minute amounts of copper as

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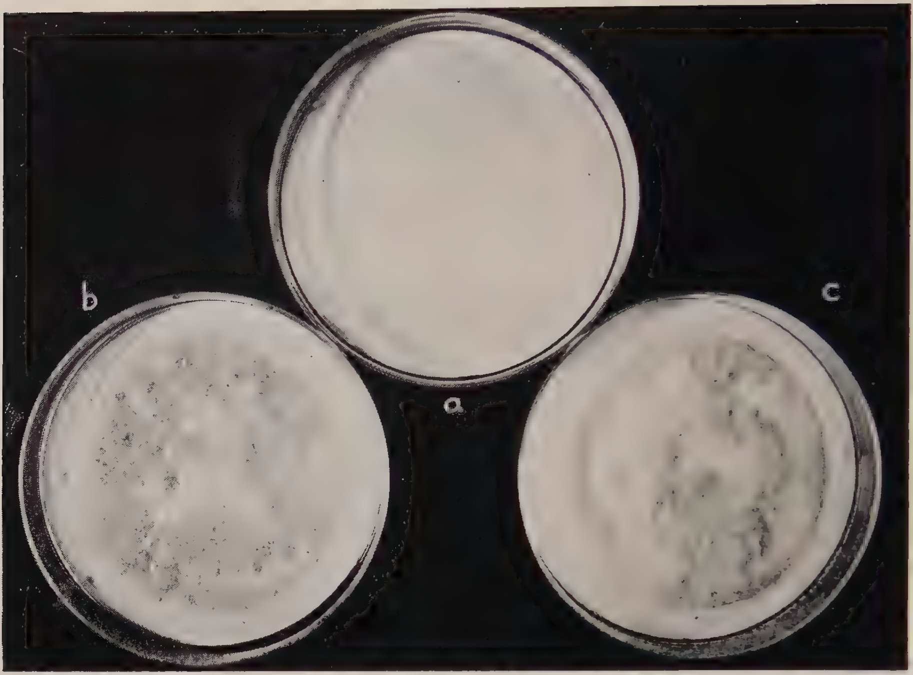
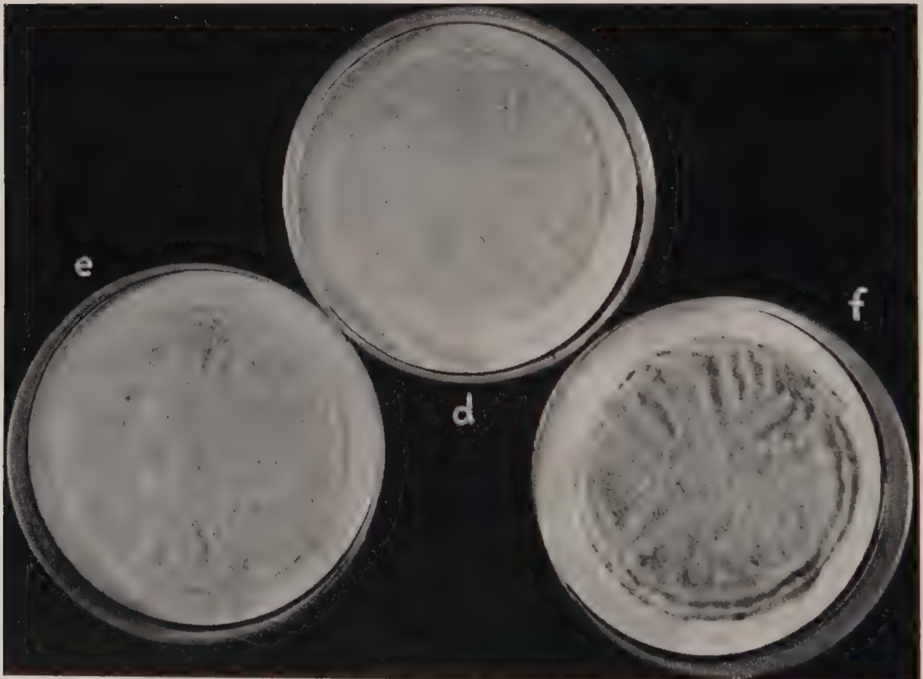
THE USE OF *Aspergillus niger* (M) FOR THE DERTERMINATION OF MAGNESIUM, ZINC, COPPER AND MOLYBDENUM AVAILABLE IN SOILS TO CROP PLANTS. D. J. D. NICHOLAS AND A. H. FIELDING.

PLATE I





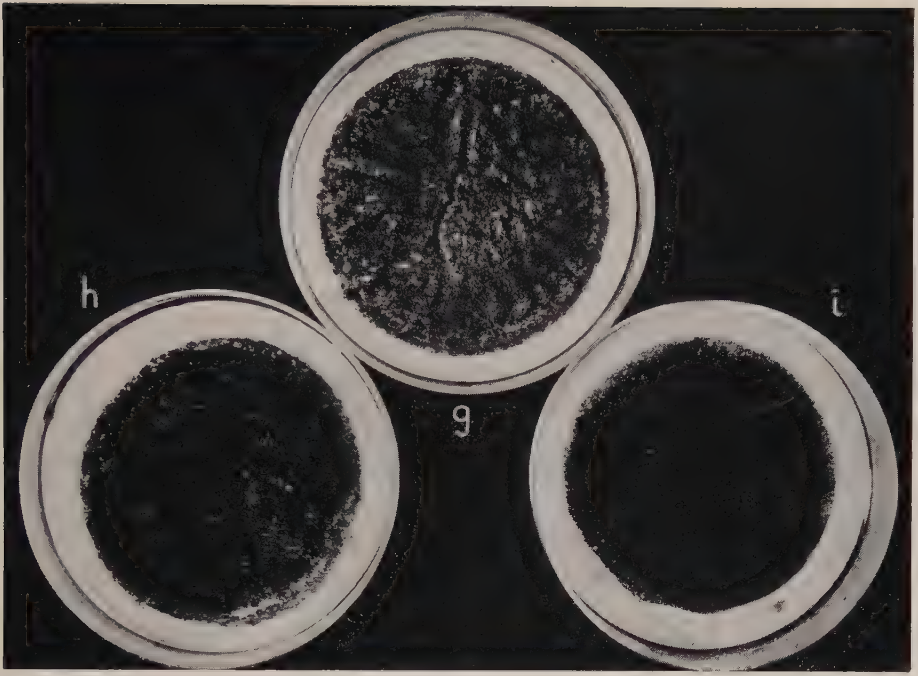


FIG. 1.

Standard series for Mg.—Aspergillus niger.

Growth of *Aspergillus niger* after six days at 25° C. in culture solutions containing the following amounts of Mg in μg per 50 ml. culture solution respectively: (*a*) 0, (*b*) 25, (*c*) 50, (*d*) 100, (*e*) 150, (*f*) 200, (*g*) 300, (*h*) 400 and (*i*) 500.

PLATE II

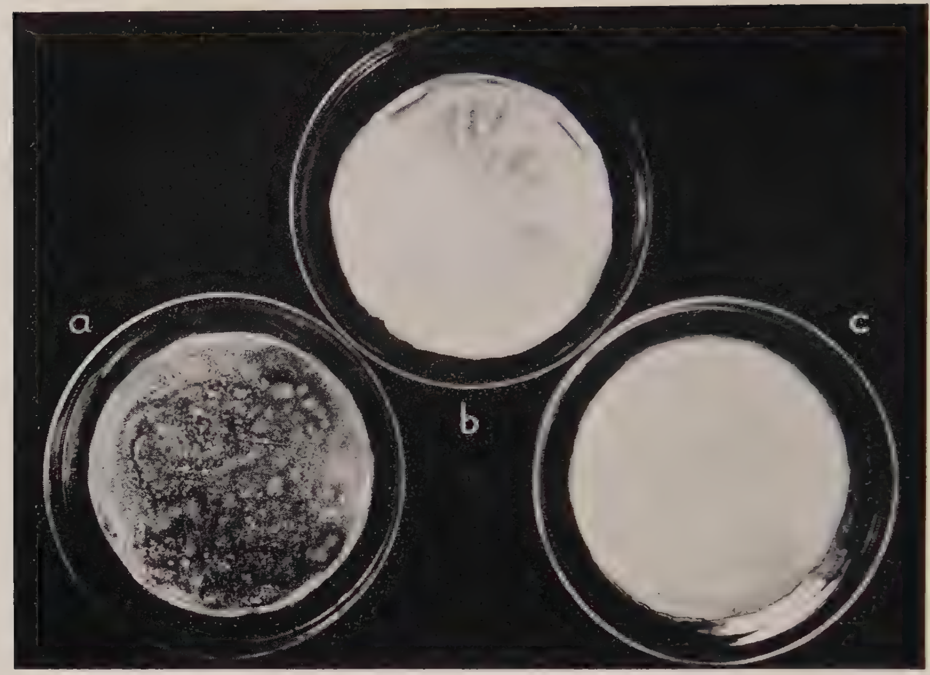


FIG. 2.

Bioassay of Mg in soils—Aspergillus niger.

(*a*) Soil with adequate Mg (NPK, Mg treatment) : 300 μg Mg per gm. air dry soil
(*b*) Soil deficient in Mg (NPK treatment) : 150 μg Mg per gm. air dry soil.
(*c*) Soil deficient in Mg and K (NP treatment) : 150 μg Mg per gm. air dry soil.

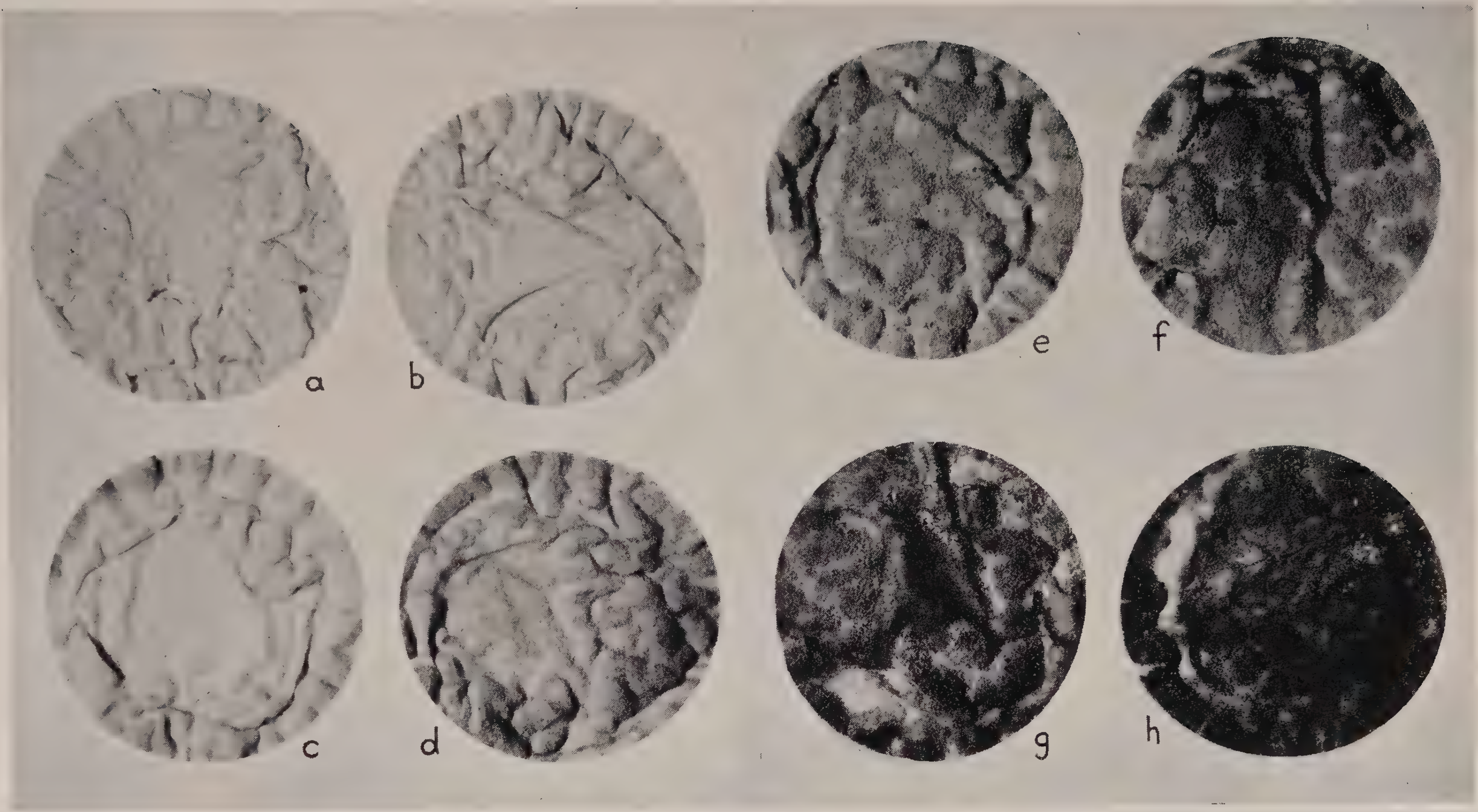


FIG 3.

Standard series for Cu—Aspergillus niger.

Growth of *Aspergillus niger* after 6 days at 25° C. in culture solutions containing the following amounts of Cu in μg per 50 ml. culture solution respectively: (*a*) 0, (*b*) 0·1, (*c*) 0·2, (*d*) 0·4, (*e*) 0·6, (*f*) 0·8, (*g*) 1·0, (*h*) 2·0.

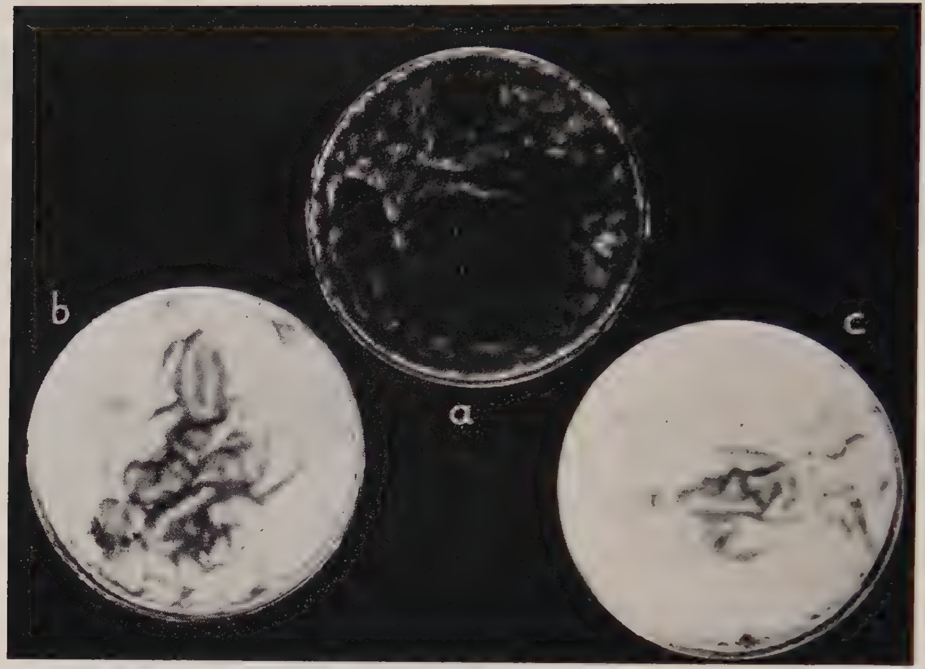


FIG 4

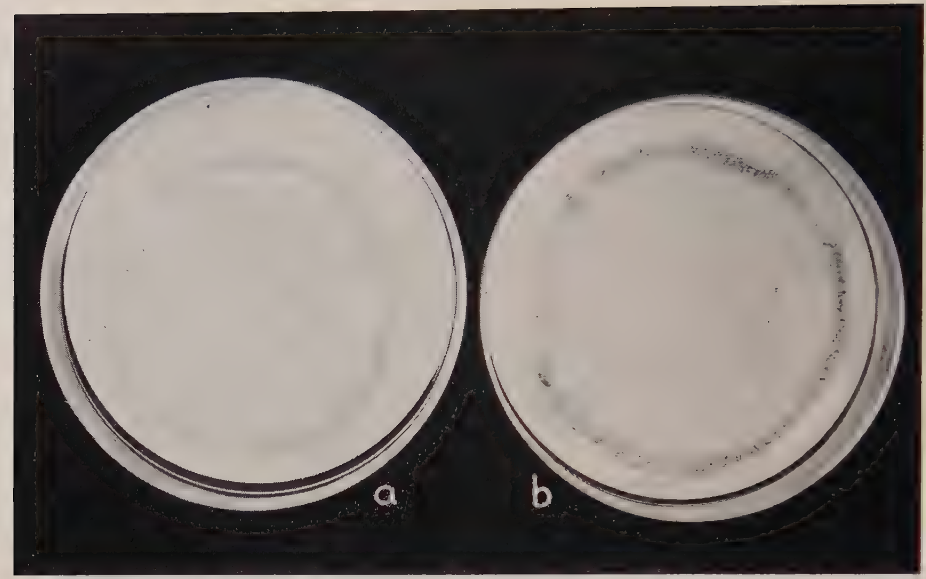
Bioassay of Cu in soils—Aspergillus niger.

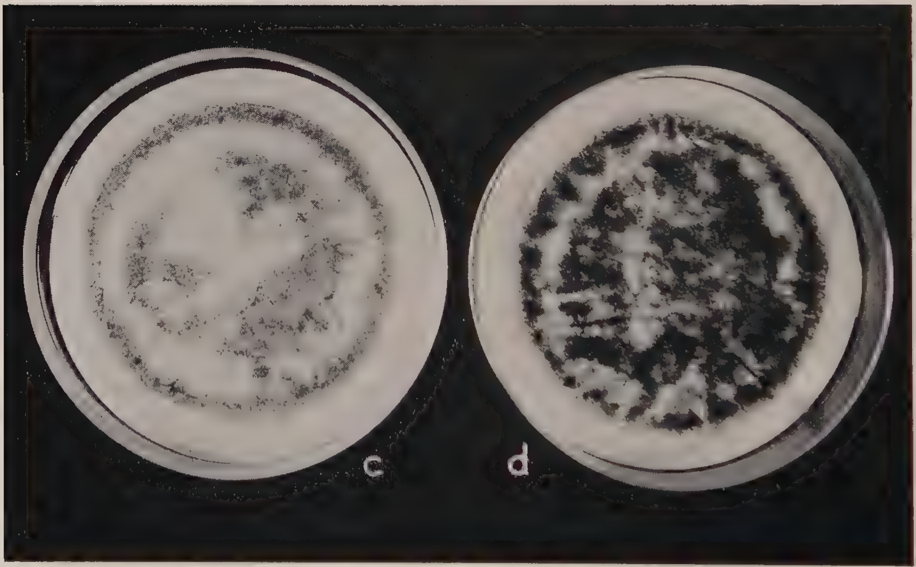
(*a*) Soil from Long Ashton with adequate Cu top and subsoil (0–6″ and 6–12″) : 25 μg Cu per gm. air dry soil.

(*b*) Surface soil (0–6″) from Wisley, Surrey, showing a deficiency of Cu : 0·6 μg Cu per gm. air dry soil.

(*c*) Subsoil (6–12″) from Wisley, Surrey, showing a deficiency of Cu : 0·2 μg Cu per gm. air dry soil.

PLATE V





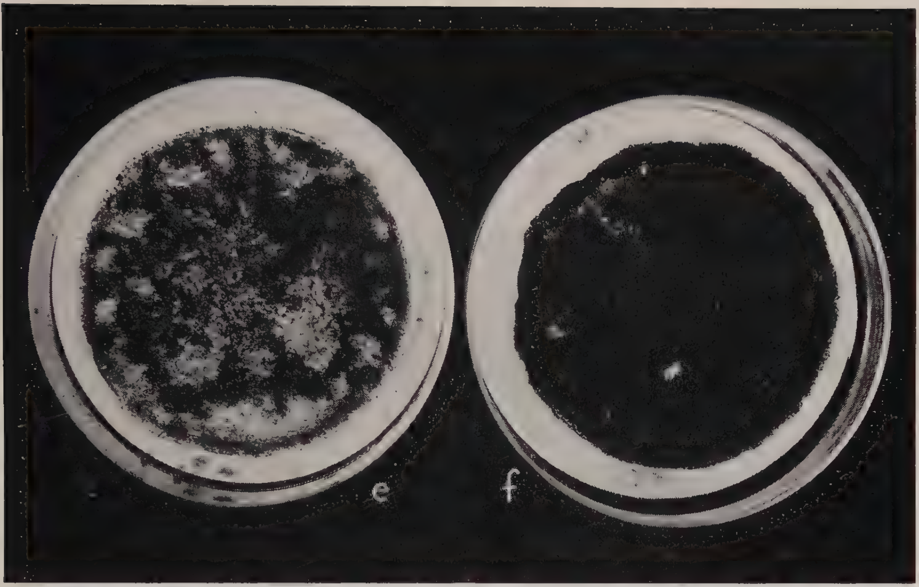


FIG. 5.

Standard series for Zn—Aspergillus niger.

Growth of *Aspergillus niger* after six days at 25° C. in culture solutions containing the following amounts of Zn in μg per 50 ml. culture solution respectively: (*a*) 0, (*b*) 1, (*c*) 2, (*d*) 3, (*e*) 4, (*f*) 8.

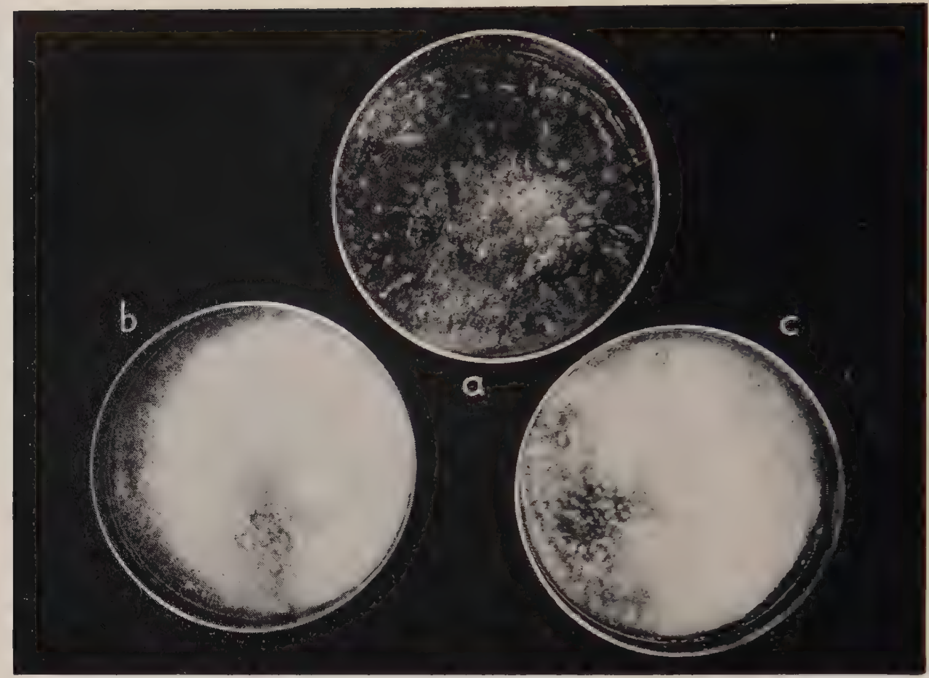


FIG. 6

Bioassay of Zn in soils—Aspergillus niger.

(*a*) Long Ashton soil with adequate Zn top and subsoil (0-6 in. and 6-12 in.) : 20 μg Zn per gm. air dry soil.

(*b*) Surface soil (0-6 in.) from Wisley, Surrey, showing a deficiency of Zn: 4 μg Zn per gm. air dry soil.

(*c*) Subsoil (6-12 in.) from Wisley, Surrey, showing a deficiency of Zn: 4 μg Zn per gm. air dry soil.

PLATE VII

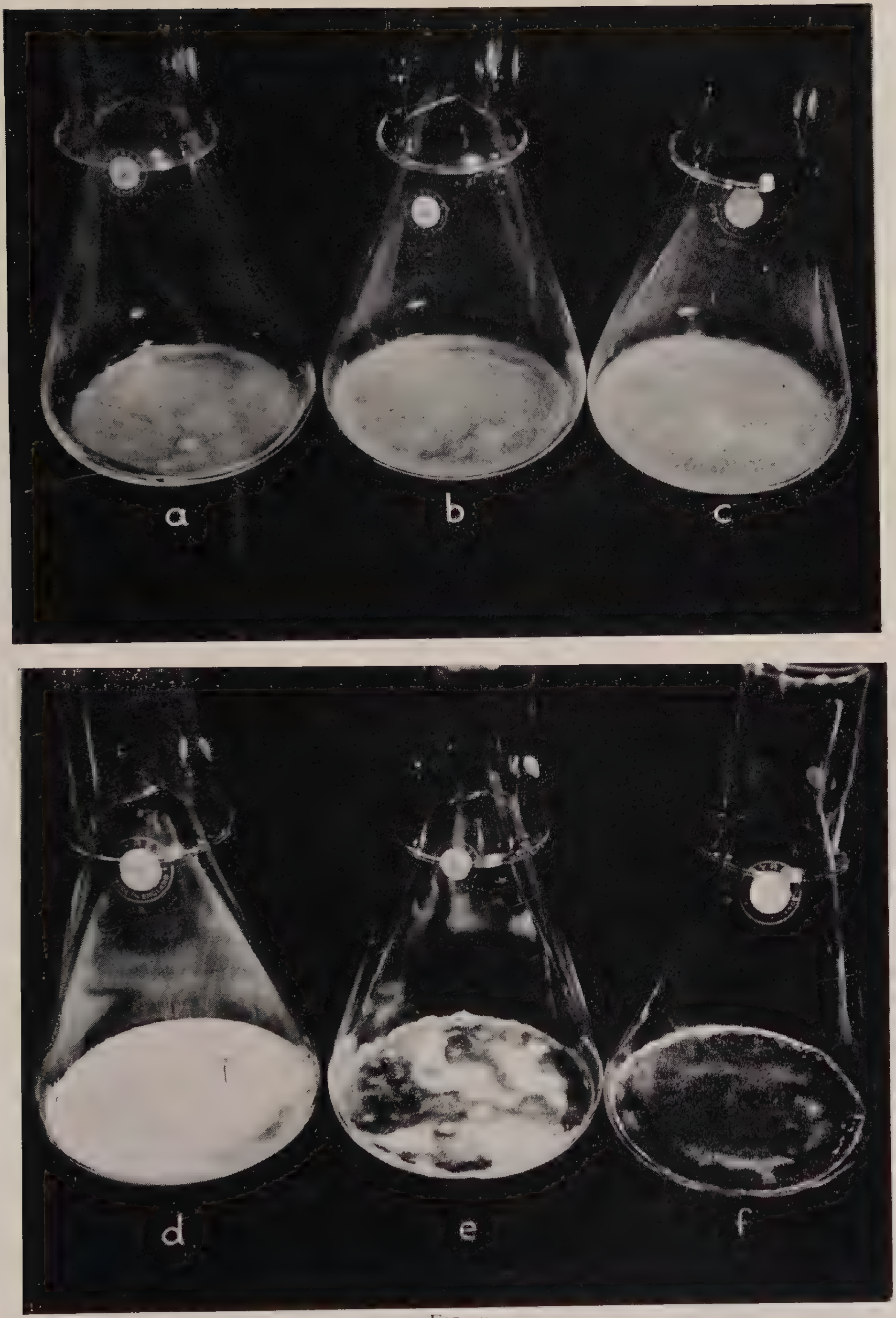


FIG. 7.

Standard series for Mo.

Growth of *Aspergillus niger* after six days at 25° C. in culture solutions containing the following amounts of Mo in μg x 10^{-4} per 50 ml. culture solution: (*a*) 0, (*b*) 5, (*c*) 10, (*d*) 50, (*e*) 100, (*f*) 500.

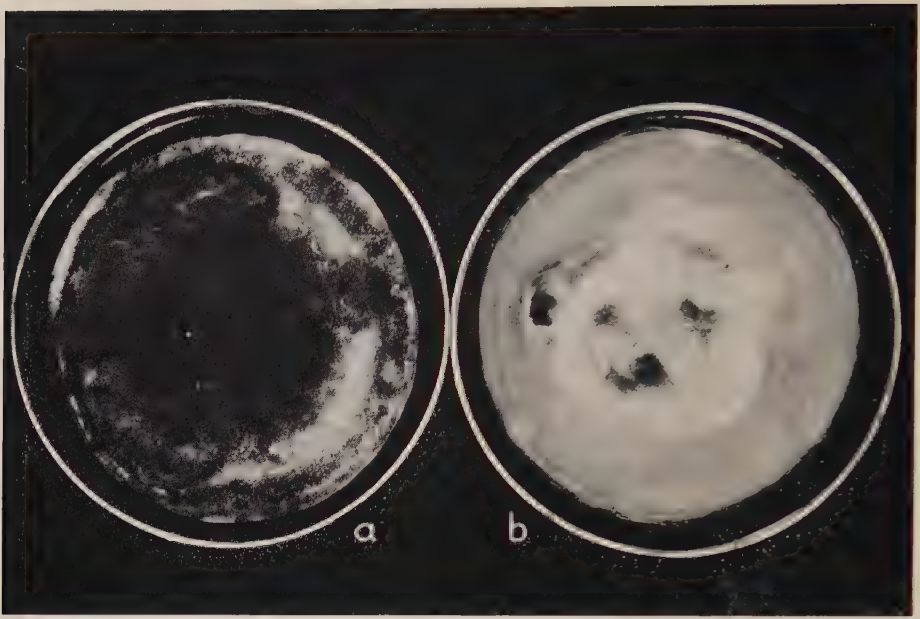


FIG. 8.

Bioassay of Mo in soils—Aspergillus niger.

(*a*) Long Ashton soil with adequate Mo : $>0\cdot5$ μg per gm. air dry soil.
(*b*) Soil from " whiptail " area in Wilts deficient in Mo : $0\cdot05$ μg per gm. air dry soil.

TABLE IV.

Relation between visual symptoms, plant analysis, chemical and bioassay data for Mg in soils.

Manurial treatment.	Block.	Visual deficiency symptoms.	PLANT ANALYSIS. Potato (mid-stem leaves) % dry matter.		SOIL ANALYSIS as % air dry soil. Bioassay. 1 gm. soil pre-treated at 200° C. for 1 hr.	1 gm. soil autoclaved with culture solution.	Chemical (Exchangeable).	
			K_2O	MgO	Mg.	Mg.	Mg.	K
N.P.K. ..	I	Mg. (trace)	4·35	0·55	0·006	0·009	0·004	0·027
	II*	nil	4·72	0·96	0·016	0·016	0·014	0·025
	III	Mg. (trace)	4·69	0·52	0·007	0·006	0·005	0·029
PK (omit N)	I	Mg. (slight)	5·42	0·40	0·005	0·007	0·004	0·035
	II*	nil	4·15	0·92	0·016	0·014	0·014	0·030
	III	Mg. (medium)	6·11	0·25	0·006	0·007	0·004	0·037
NP (omit K)	I	K (severe)	0·63	1·16	0·006	0·005	0·003	0·003
	II*	K (severe)	0·86	1·46	0·013	0·014	0·015	0·005
	III	K (severe)	0·77	1·06	0·007	0·008	0·006	0·005

*Magnesium sulphate applied to all plots in this Block.

Statistical analysis of bioassay data: P = 0·001. Block II > **Blocks** I and III, no significant differences between treatments.

	Mean.	*S.D.*
Block I and III	0·005	± 0·001
Block II	0·014	± 0·003

TABLE V.

The growth of Aspergillus niger in relation to copper content of the culture solution. (Grown at 25° C. for six days.)

Cu as µg. in 50 ml. culture solution.	Description of mycelium and spore cover.		Dry weights of fungus in mg.	pH of culture solution after growth.	Designation in Fig. 3.
	Colour of spores.*	Spore cover.			
0	White	Complete absence of spores	215·8	8·24	a
0·05	Pale pinkish buff	Few spores	237·6	8·34	
0·1	Pinkish buff	Spores sparsely distributed	301·6	8·04	b
0·2	Cinnamon buff	Spores covering parts of mycelium	384·9	6·56	c
0·3	Clay colour	Spores becoming more profuse	440·0	6·92	
0·4	Light tawny olive	,, ,,	454·2	6·98	d
0·5	Tawny olive	Spores covering most of mycelium	476·8	6·78	
0·75	Saccardo's umber	,, ,,	642·5	5·93	
1·0	Sepia	Spores completely covering mycelium	726·0	4·33	g
2·0	Black	,, ,,	758·4	4·30	h
2·5	Black	,, ,,	868·5	6·86	
full culture = 12 µg	Black	,, ,,	969·0	2·94	

* The colours of spores are referred to Ridgeway's Colour Standards, Plate 29, 17 in. O-Y.

shown in Table V. The dry weights of fungus increase with ascending Cu values and it is noteworthy that complete black spore cover does not necessarily coincide with maximum growth. The pH of the culture solution after growth varies from 8·24 to 2·94 at nil and 12 µg Cu respectively. This reflects the production of organic acid (mainly citric acid) by the fungus which is usually at a maximum at optimum nutrition, viz. full culture.

Bioassay of soil. Soil thoroughly dried in air was taken for the bioassay of copper, and 0·1, 0·25, and 0·5 gm. portions respectively were used depending on the Cu status. For normal soils 0·1 or 0·25 gm. was adequate but for soils very deficient in the micronutrient 0·5 gm. was used.

Soils from alkaline fen soils in East Anglia and sandy soils from S.E. England were compared with those from Long Ashton and the results are given in Table VI.

TABLE VI.

Relation between visual symptoms and Cu levels in crops and soils.

Location.	Soil characters.	Visual symptoms in crops.	Analysis of plant material Cu as μg. per gm. dry matter.			Bioassay of Cu in soil as μg. per gm. air dry soil.	
			Cereals.		Apple leaves.		
			Straw.	Ear.		0-6″	6-12″
Long Ashton, Somerset	Calcareous sandy loam overlying Keuper Marl, pH 7·2	No Cu deficiency	10 to 18	7 to 12	> 5	50	15 to 20
Methwold Fen, Norfolk	Alkaline fen peat, with a high water table, pH 7·6	*Cu deficiency in cereals.* Malformation of the ears "withertip". Younger leaves spirally distorted. Marked reduction in yields	4 to 6	5	—	2·6	0·5
Wisley, Surrey ..	Sandy soil, overlying coarse river gravel. pH after liming, 7·0	*Cu deficiency in fruit trees.* Malformation of younger leaves resulting in defoliation and die-back of the leader shoots	—	—	1 to 3	1·3	0·33

Statistical analysis of bioassay data for soils :
P = 0·001 Long Ashton > Methwold and Wisley soils.

	Mean.	*S.D.*
Long Ashton	50	± 10
Methwold	2·58	± 0·274
Wisley 0-6″	1·29	± 0·40
Wisley 6-12″	0·33	± 0·20

Certain alkaline fen soils in East Anglia, e.g. Methwold Fen, are not only low in available Mn but also in Cu. Cereal crops grown in one of these soils during 1946 showed symptoms of Cu deficiency, and the application of 50 lb. per acre of copper sulphate to the soil resulted in increased yields. During 1947 foliage spray treatments incorporating various combinations of dilute solutions of Mn, Zn, Cu, Ni and Co as sulphates (at 0·25 per cent.) showed that Cu had a significant effect in correcting the deficiency and in increasing yields.

The bioassay values for Methwold and Long Ashton surface soils, viz. 0-6 in. are 2·6 and 50 μg Cu respectively. These highly significant differences in the Cu status of the soils (see Table VI) are in agreement with the Cu content of the cereal crops grown at both centres. The Cu content of the cereal (straw and ear respectively) confirm a deficiency of the element at Methwold. It is noteworthy that in 1946 and 1947 after recent ploughing from turf, the deficiency was more marked than in 1949, and bioassay of the soil shows that levels of available Cu have increased annually. This may be related to a decrease in organic matter of the soil.

The bioassay method shows that the sandy soil at Wisley is low in available Cu, viz. 0·3 to 0·6 μg. per gm. air dry soil (Fig. 4). This confirms symptoms of the deficiency in apple previously reported at this centre (Bould *et al.*, 1950).

The bioassay values for Cu in the deficient soils are in agreement with those obtained by Mulder (1939) in Dutch soils where " reclamation disease " is a problem

TABLE VII.

Relation between Zn content of culture solution and growth of Aspergillus niger. (Grown at 25° C. for six days.)

μg. Zn in 50 ml. culture solution.	Description of mycelium and spore cover.	Dry weights of fungus in mg.	pH of culture solution after growth.	Designation in Fig. 5.
0	Thin entirely mucous wefts of mycelium. No spores	113·1	3·26	a
1	Thin entirely mucous. Few black spores around periphery	275·8	3·23	b
2	Thicker mycelium : scant cover of black spores	314·8	3·30	c
3	Greater profusion of black spores	452·6	3·41	d
4	Black spores nearly covering the thick mycelium	545·9	3·51	e
5	Black spores completely covering mycelium	644·1	3·59	
7·5	Denser mat of black spores, very thick undulating mycelium	844·6	3·82	
10	Denser mat of black spores, very thick undulating mycelium	894·0	4·19	f

and those reported by Acock (1941) for Cu deficient soils in South and West Australia. Values between 0·5 and 2 μg. per gm. soil were regarded as threshold of deficiency levels for Cu likely to produce a deficiency in higher plants and these are confirmed in the British soils. Normal soils, however, appear to contain more copper than did those assayed by Mulder and Acock respectively. This may be due to the fact that the two authors use one gram portions for assay irrespective of status, so that values obtained for normal soils are determined at the extreme end of the growth curve where it is least sensitive.

Zinc.

Standard series. A standard growth series for Zn is given in Table VII and Fig. 5.

A comparison between the Cu (Table V) and Zn standard series shows the following points of difference : in the absence of Zn the mycelium is entirely mucous and the dry weight of fungus is lower than in the absence of Cu ; there is no spore colour change in the Zn series as shown for Cu ; more Zn is required for optimum growth than Cu ; there is no marked change in pH values of culture solution as in the Cu series indicating that organic acids are not produced by the fungus when Cu is deficient. It is noteworthy that at 5 μg. Zn there is complete sporulation and that using spore density as a criterion for assay, it is difficult to determine higher Zn values. Dry weight values, however, increase up to 10 μg. Zn. It is essential to use the 1 to 4 μg. Zn range so that sporulation and dry weights may be used in complementary roles for the bioassay of the micronutrient. A similar phenomenon was noted in the Cu standard series.

Bioassay of soil. Air dry soil was used for bioassay of Zn and 0·25 or 0·5 gm. portions of normal soils and of those likely to be deficient in the micronutrient, respectively, were tested.

TABLE VIII.

Relation between visual symptoms and Zn status of apple trees and soils.

Location.	Soil characters.	Visual symptoms in fruit trees.	Analysis of apple leaves and bark. Zn as μg/gm. dry matter.		Bioassay of Zn in soil as μg. per gm. air dry soil.	
			Leaves.	Bark.	0-6″	6-12″
Long Ashton, Somerset	As in Table V	No Zn deficiency	10 to 20	15 to 20	30 to 40	20 to 25
Wisley, Surrey	As in Table V	Cu deficiency (see Table V). Zn deficiency "little leaf" and rosetting of leaves	1 to 2	1 to 2	5 to 8	3

Statistical analysis of bioassay data for soils :
P = 0·001 Long Ashton > Wisley.

	Mean.	*S.D.*
Long Ashton	32 ±	8·5
Wisley	8 ±	1·1

Test samples were taken from sandy soils located at Wisley in Surrey and compared with Long Ashton soils. The data are shown in Table VIII and illustrated in Fig. 6.

Zinc deficiency in fruit trees at Wisley diagnosed by visual symptoms and chemical analysis of leaves and bark tissues from normal and affected trees (Bould *et al.*, 1949) is confirmed by the bioassay values for Zn in the soils. The data in Table VIII show highly significant differences in available Zn between Long Ashton soils and those from Wisley. Zinc values for normal soils are variable and are usually over 10μg. Zn. The highest value so far recorded is 40 μg. Zn per gm.

It is noteworthy that Cu, Zn, and Mg deficiencies occur together in fruit trees at Wisley and bioassay of soils shows low values for all three nutrients.

Molybdenum.

Standard series. Data for the Mo standard growth series are shown in Table IX and Fig. 7.

TABLE IX.

The growth of Aspergillus niger in relation to the Mo status of the culture solution. (Grown at 25° C. for six days at pH 2·0.)

μg. Mo in 50 ml. culture solution.	Description of mycelium and spore cover.	Dry weights of fungus in mg.	pH of culture solution after incubation.	Designation in Fig. 7.
0	Thin strands of broken mycelium entirely mucous	57	2·56	a
0·0001	Thin strands woven into a thin pellicle, entirely mucous	114	2·92	
0·0005	Thin strands woven into a thin pellicle, entirely mucous	173	3·49	b
0·001	Thicker pellicle entirely mucous	275	3·58	c
0·002	Thicker pellicle entirely mucous	400	3·88	
0·005	Occasional black spores on small islands of white hyphal growths alternating with mucous areas	537	1·67	d
0·01	Scant sporulation, thicker mycelium	638	2·18	e
0·02	Abundant spores covering thick undulating mycelium	795	2·19	
0·05	Complete black spore cover, very thick mycelium	950	2·10	f
30	Complete black spore cover, very thick mycelium	988	2·00	

Bioassay of soil and plant tissue. The pH of the normal culture solution approximates to 4, and using as little as 0·1 gm. of soil for test it is not possible to show differences between Mo levels at this pH. This is due to the extreme sensitivity of the method, since as little as 0·02 μg. Mo is adequate for optimum growth. As Mo salts are less soluble in acid than in alkaline solution, the pH of the culture solution was adjusted to 1, 1·5, 2, 2·5 and 3 respectively. At each pH level, triplicate soil samples were taken from normal soil (Long Ashton) and acid soils likely to be low in available Mo, using 0·05, 0·1, 0·5 and 1 gm. respectively. It was found that 0·5 gm. or 1 gm. of soil at pH 2 gives the maximum difference between the normal soils and those from special centres, where " whiptail " symptoms occur in cauliflower (Hewitt *et al.*, 1947, Waring and Wilson, 1949). Below pH 2, in the absence of soil, the growth of the fungus was retarded by the acidity of the culture solution. In the presence of 0·5 gm. of soil, however, the fungus grew well even at pH 1.

The data for the bioassay of Mo in soils and comparison with the results of other diagnostic methods are shown in Table X and Fig. 8.

TABLE X.

Relation between visual symptoms and Mo status of cauliflower and soils.

Location.	Soil characters.	Visual symptoms in cauliflower.	Analysis of cauliflower leaves. Mo as μg. per gm. dry weight.	Bioassay of Mo in soil as μg. per gm. air dry soil.
Long Ashton, Somerset	Sandy loam, calcareous	No " Whiptail "	1 to 5	0·5
Bromham, Wiltshire	Light sand, acid	Severe " Whiptail"	0·05 to 0·12	0·01
St. Ives, Cornwall	Shaley loam, acid	Severe " Whiptail"	0·01 to 0·08	0·015 to 0·03

Statistical analysis of bioassay data:
P=0·001 Long Ashton > Bromham and St. Ives.

	Mean.	*S.D.*
Long Ashton	0·5	± 0·005
Bromham	0·01	± 0·002
St. Ives.	0·02	± 0·002

It is clear that there are significant differences between the available Mo in the Long Ashton soil and in those from the centres where " whiptail " symptoms appear in cauliflower. The soil tests for Mo are paralleled by similar trends for the micronutrient in leaf analysis of normal and affected cauliflower plants.

DISCUSSION.

The use of *Aspergillus niger* for the bioassay of Mg, Cu, Fe, Zn, Mo and Mn respectively presents problems in the preparation of culture solutions free from these nutrients. The diversity of methods used to eliminate them, including the use of both chemical and ion exchange techniques, emphasizes the complexity of the problem associated with the preparing of media for pure culture work. Moreover when chemical methods fail to detect a given nutrient in a culture solution there may be sufficient present to support adequate growth of *Aspergillus niger*. This is well shown in the case of Mo, as the fungus can detect differences of 0·0001 μg. whereas the chemical limit of detection is 0·1 μg. Mo per 50 ml. respectively. Thus the efficacy of removal of micronutrients can only be satisfactorily determined using the bioassay procedure.

The extreme sensitivity of the method for the determination of micronutrients necessitates the use of stringent precautions in preparing glassware, distilled water and culture solutions so that satisfactory responses to additions of the test element can be determined. The effective ranges in μg. per 50 ml. culture solution emphasize this point ; Mg, 25 to 500 ; Cu, 0·05 to 2 ; Zn, 0·1 to 10 ; Fe, 0·1 to 20 ; Mn, 0·01 to 10 ; Mo, 0·00005 to 0·02.

The absences of Mg, Zn, Fe and Mo respectively resulted in thin wefts of mucous mycelia devoid of spores, whereas the omission of Cu or Mn did not inhibit mycelium growth to the same extent. This may be due to the fact that copper and manganese are not essential for the production of a minimum weight of mycelium or to the purification methods used being insufficiently refined to reduce mycelium growth further. All the nutrients mentioned affect sporulation and in addition Cu appears to have a specific effect on the pigmentation of the spores, a property used by Mulder (1939) to indicate Cu level. The intensity of spore cover and dry weights of fungus are used in complementary roles for the bioassay of the nutrients. It is found, however, that adequate sporulation may not coincide with maximum dry weight yields of the fungus which may increase with added " test " nutrient when complete spore cover is achieved at lower levels. It is thus important to attempt assays on parts of the growth curves where dry weights and intensity of sporulation may be used together as indices of the nutrient assayed. In making assays of soils, e.g. for Cu, intensity of sporulation may be a better criterion of level than is the dry weight of fungus.

It is realized that soils are heterogeneous and are composed of organic complexes, in addition to the inorganic fractions. Despite this, the bioassay results appeared to be specific for the "test" nutrient determined in the soil and were not influenced by other soil factors. The addition of a variety of organic constituents, including amino acids, growth promoting substances and humic acids, did not appear to affect the specificity of the response to the particular mineral element studied.

Biological contamination from other micro organisms present in soil samples was overcome by pretreating the soils before assay. When one-gram samples were used for Mg assay, it was found necessary either to autoclave the soil with the culture solution or to preheat it in an oven before assay. It is well known that pre-treatment of soil in this way may release nutrients (Russell, 1932), but differences in Mg values between normal soils and those deficient in Mg were maintained after heat treatment. For the assay of micronutrients, the smaller amounts of air dry soil used did not give rise to any serious contamination problems.

It is known that the availability of micronutrients to crop plants is related to the pH of the soil. Thus, it is important to determine the pH of the culture solution and the shift likely to occur after incubation in the presence of soil. The small quantities of soil used did not appear to affect the pH of the buffered culture solution appreciably, but the production of citric acid by the fungus produced large effects on the acidity of the solution. It may be necessary to adjust the pH of the culture solution at the outset, so as to control the release of mineral nutrient to be assayed from the soil. This is important for Mo, as sufficient of the micronutrient is available to the fungus when 0·5 gm. samples of normal or Mo deficient soils are incubated in the culture solution at approximately pH 4. Significant differences are shown between normal and Mo deficient soils when the pH of the culture solution is adjusted to 2 before addition of the soil for test.

The determination of micronutrients in soils using chemical procedures involves the use of extractants which may not reproduce the extracting properties of the roots of plants. Moreover the quantities extracted are small, so that subsequent chemical determinations may be difficult. The advantages of the bioassay method include a micro-biological determination of the mineral nutrients, which seems to be in keeping with the availability of the nutrient to the higher plants, and the extreme sensitivity and specificity of the method for their determination. Thus Cu deficiency has been confirmed in alkaline fen soils, where cereal crops showed symptoms of the deficiency, Cu and Zn deficiencies in a sandy soil, where fruit trees were affected, and Mo deficiency in soils, where brassica plants showed " whiptail " symptoms (Hewitt *et al.*, 1947, Waring and Wilson, 1949). Further, by means of the bioassay method, a deficiency of Mg can be detected in the soil in the presence of K deficiency. This may not be shown by a chemical analysis of the crop plants growing therein, because of the interaction between the two nutrients. Thus when K is low in the plant tissues, Mg may be high, even on soils deficient in the element.

The main disadvantages of the bioassay method are those usually associated with the use of biological material, viz. the variation in results using different strains of the organism, the possible mutation within a given strain leading to a different response and the problem of culturing the fungus. Such changes in behaviour can be detected by the frequent checking of standard series for the " test " nutrients.

In the part of the investigation described in this paper only one strain of *Aspergillus niger* has been examined and it is clear that there may be other fungi and bacteria equally or perhaps more sensitive as test organisms for the bioassay of micronutrients. An extension of this work to other such organisms is in progress.

SUMMARY.

1. *Aspergillus niger* (M) has been used as a test organism for the determination of magnesium, copper, iron, zinc, manganese and molybdenum respectively in test materials. The response of the organism to these nutrients respectively is specific and quantitative.

2. Methods have been developed for the preparation of culture solutions biologically free from magnesium, copper, iron, zinc, manganese, molybdenum and gallium respectively. These include chemical co-precipitation procedures as well as ion exchange methods.

3. The extreme sensitivity of the bioassay method is shown by the following effective nutrient ranges in μg per 50 ml. culture solution ; Mg, 25 to 500 ; Cu, 0·05 to 2 ; Zn, 0·1 to 5 ; Fe, 0·1 to 20 ; Mn, 0·01 to 10 ; Mo, 0·00005 to 0·02.

4. Dry weight yields of fungus and intensity of sporulation are used in complementary roles for the determination of a response to a nutrient under test. The two methods of evaluation do not always give parallel results, as it is possible to get a complete sporulation without having attained maximum dry weight and vice versa. For assays of zinc and molybdenum, dry weights are important and, for copper, spore colour is a better index of nutrient level.

5. Air dry soil was used for bioassay work. Interference from other micro-organisms was sometimes encountered especially when one gram portions of soil were used for the assay of Mg. In these instances, treatment of soil by autoclaving with the media, or preheating, eliminated foreign fungi.

6. The pH of the culture solution had an important effect on the availability of nutrients. For Mo, the pH must be adjusted to 2, so as to get the maximum difference between normal and soils deficient in the element.

7. Using the bioassay method for soils, the following deficiencies were confirmed: magnesium in the presence of potassium deficiency; copper deficiency in alkaline fen soils and a sandy soil, and also zinc deficiency in the latter; molybdenum deficiency in soils where brassica plants showed "whiptail" symptoms. The assay results agreed with crop performance at the various centres.

8. One of the advantages of the bioassay method over chemical procedures for the determination of mineral micronutrients in soils, is its sensitivity; in some instances the amounts determined are below the limit of chemical detection.

9. It is suggested that the use of other micro-organisms, including fungi and bacteria, merits investigation.

ACKNOWLEDGMENTS.

The work described herein was financed by the Agricultural Research Council to whom grateful acknowledgment is made. We are grateful to Prof. T. Wallace and to Mr. R. W. Marsh for useful discussion.

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(*Received* 18/5/50.)

THE INTERNAL BLACKENING OF POTATOES CAUSED BY BRUISING

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MANY different types of internal blackening and necrosis may be found in potato tubers and it is often rather confusing to try to distinguish the separate symptoms, especially in view of the fact that as yet the cause of some of them is obscure. Excluding the blackening which often occurs after cooking and the internal necroses known as spraing, internal rust spot and net necrosis which are frequently obvious when the tubers are lifted, there are other types which involve blackening of the tissue rather than necrosis and which are often due mainly to conditions during storage. For example, extremes of temperature may cause discoloration of the tubers, chilling often causing a blackening or discoloration of various types, while an excessively high temperature or lack of aeration may produce the condition known as Blackheart.

The condition of blackening of potato tissues known as " bruise " appears to have been described first by Horne (1913) in England, who found that the phenomenon was fairly frequent, especially late in the storage season, e.g. in May. He found no bacteria or fungal hyphae in the affected tissues nor could any transmission through seed tubers be effected and he considered it to be physiological in origin. He stated that although it was commonly called " bruise ", it was not caused by any external pressure or blow received, for instance, by tubers falling on the ground ; he noted two cases, observed in January, 1909, where the tubers were badly blackened and showed rather different general symptoms. In the present experiments no examples of extensive blackening have been observed so early in the storage season and circumstantial evidence would lead to the conclusion that in these two cases the cause may have been severe chilling.

Several Dutch workers, Botjes (1927, 1929), van der Waal (1929) and de Bruyn (1929) investigated the condition described as " blueing " or " blue spotting " and associated this with the bruising more particularly of potash deficient tubers which had lost turgidity. They were able to reproduce the condition by allowing the tubers to fall from various heights. Botjes (1929) found that the blackening, due to lifting injury, could develop some weeks after harvest, while McIntosh (1935) and Scott (1936) in this country also obtained discoloration in tubers bruised not long after harvest. The former observed that it occurred to a greater extent in those which remained uncracked since such tubers would probably sustain greater disruption of the internal tissues, but the latter obtained fairly extensive blackening even in those which were cracked. From the present investigation, however, it would appear that normal damage early in the storage season does not have such serious effects as that later in the season, e.g. after sprouting commences. Koblet (1947) in Switzerland records internal blackening after tubers had been desprouted in July, and this was increased if extra care was not taken.

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The blackening of the tubers which occurs after bruising may be confused often with that due to chilling or frost or sometimes in severe cases with that of Blackheart. It is observed most frequently late in the storage season, e.g. from April onwards and the affected tubers are usually in a condition of reduced turgidity. On cutting the tubers (Fig. 2) the flesh shows a grey to black discoloration usually at or near the site of the bruise which may not be visible externally. The discoloration commences round the vascular ring and extends mainly inwards leaving the cortex and often the central medullary region relatively unaffected. Severely affected tubers may show almost complete internal blackening and such tubers frequently shrivel to such an extent as to become quite hard and mummified (Fig. 1). In these cases the cell walls in the blackened tissues disintegrate leaving merely a consolidated mass of starch. Extensive shrinking of the blackened area occurs when the cut surface is exposed for any length of time.

The results of the present investigation into the cause of this blackening and some of the contributory conditions show that the discoloration depends on two main factors : *A* the condition of the tuber ; *B* the degree of bruising. To these must be added another factor, *C* which was found by several Dutch workers, i.e. conditions of growth involving lack of potash or excess of nitrogen.

A. Condition of the tuber.

The tuber must be in a relatively flaccid condition and any factor which favours loss of turgidity will render the tuber more susceptible to the effects of bruising.

1. *Temperature of storage.* Part of a stock of Majestic tubers was kept in a potato store at a temperature which varied between about 40° and 55° F. for two months (9th March to 7th May, 1945), while another part was stored in boxes at a temperature of 60° F., both these stocks being kept in darkness. Samples of 6 or 8 tubers were then allowed to fall on their sides from a height of three feet on to a concrete floor without breaking the skin, and were thereafter kept under ordinary conditions of light for a further eighteen days, when cutting revealed blackening to the extent of 100 per cent. in those originally stored at 60° F. and 50 per cent. in those stored at the lower temperature.

Again, part of a stock of Arran Pilot tubers was kept in a cold store at 38° F. from March until 14th May, 1948, at which time sprouting had not yet commenced. A similar part was kept under ordinary conditions in boxes and sprouting was very vigorous. On that date no blackening was apparent in unbruised tubers of both sets. Twenty tubers of each set were then allowed to fall four feet on to a wooden floor and while, seven days later, nineteen of the sprouted tubers showed blackening, twenty-five days later only two of the unsprouted tubers showed any discoloration on cutting.

2. *Condition of storage.* An experiment carried out with the same stock of Majestic tubers as mentioned above, in which some of the tubers had been kept in darkness and some in light under the same temperature conditions, showed that the former developed 50 per cent. blackening after being dropped from three feet, while the latter developed 33 per cent. Presumably storage in darkness which tended to increase sprouting also increased consequent moisture loss from the tuber.

Free ventilation in storage was also observed to increase susceptibility to blackening. Samples of twenty-five tubers were taken from similar positions in two clamps of Arran Peak potatoes in June, 1944, one of which had been stripped of soil three months previously, and in which the temperature was therefore probably lower than in the other clamp. It was found that, one day after desprouting the tubers, the damage so caused had brought about 70 per cent. discoloration in those from the desoiled clamp, while those from the clamp still encased in soil showed only 36 per cent.

3. *Season.* In the above-mentioned experiments, the factors involved have an effect upon the turgidity of the tubers. Consequently the conditions under which blackening can occur become more favourable as the season advances. The rapidity of production and the severity of the discoloration also increase with the season and may reach a point where an intense blackening involving practically the whole tuber may be obvious within twenty-four hours of bruising. Early in the storage season, prior to any signs of sprouting, no visible internal reaction may be seen at all. Somewhat later the symptoms may be merely a grey-blue discoloration of the flesh usually unaccompanied by cracks or a visible external bruise. As sprouting increases, the heel end of the tubers becomes more flaccid and tends to be more susceptible to bruising than the rose end. Tubers kept under ordinary conditions until May were dropped from a height of three feet on to a concrete floor at various intervals thereafter so that the place of impact was at the heel end, side and rose end, 6-10 tubers being used in each case. The tubers were cut 2-4 weeks later and the results are shown in Table I.

TABLE I.

Comparison of development of internal discoloration after bruising at different times and at different areas of the tubers.

Area of bruise.	% discoloration after bruising on different dates.		
	7th May.	24th May.	21st June.
Rose end	0	33	75
Side	20	40	70
Heel end	90	100	100

This confirms the conclusions of de Bruyn (1929) and Botjes (1929) that susceptibility was most marked at the heel end. The former worker associated this with the higher specific gravity of these tissues, but Mulder (1949) in a recent paper related it to the fact that the stem half of the tubers contains 20-40 per cent. more free tyrosin than the rose half. The results in Table I also show the increase in susceptibility of the tubers with the advance of the season.

4. *Varietal differences.* Differences in varietal susceptibility were noted in Holland by van der Waal (1929), and Botjes (1927) concluded that soft-fleshed varieties were more susceptible. Early varieties are often lifted while still immature and such tubers rapidly lose turgidity in storage. It is possible that seed from such crops kept for planting in the following year may thus become very susceptible to blackening, although this may be offset by the fact that normally such seed is boxed and is handled only at planting time. No case of blackening under these circumstances has yet been brought to the author's notice.

PLATE I.

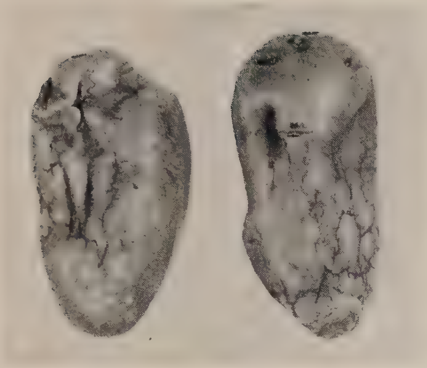

FIG. 1.

Potatoes (var. Sharpes Express) showing severe shrivelling subsequent to bruising. Such tubers also show marked internal blackening.

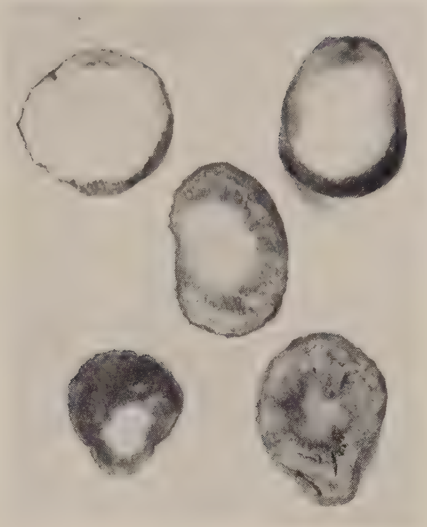

FIG. 2.

Various stages of internal blackening subsequent to bruising.

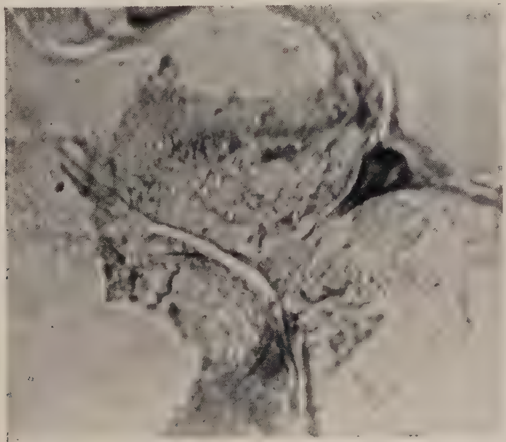

FIG. 3.

Aggregation of granules presumably of melanin in cells of the blackened tissue. × 306

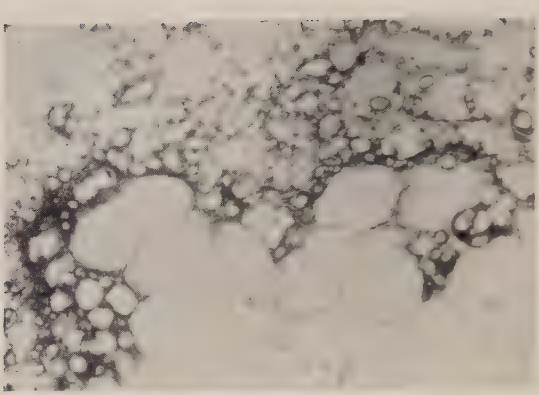

Fig. 4.

Cells at the boundary of the blackened and normal tissues in a bruised tuber showing suberization of the cell walls and starch grains embedded in the boundary layer. No wound periderm is formed. Stained with gentian violet. × 90.

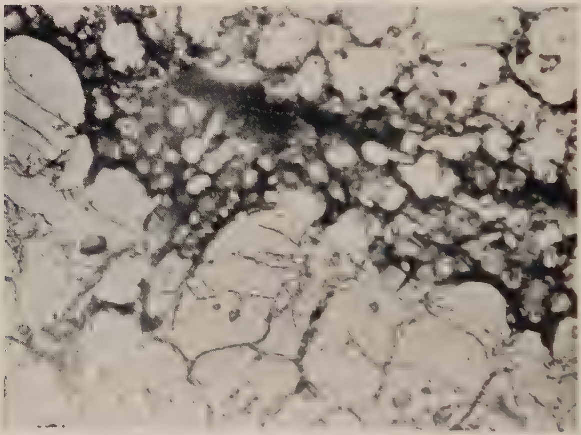

Fig. 5.

Cells at the boundary of the blackened and normal tissues in a tuber with Blackheart. The tissues are similar to those in Fig. 4, but a wound periderm is present. Stained with gentian violet. × 90.

TABLE II.

Development of internal blackening in ten varieties—February, 1950.

Variety.	Sprouting condition.	Percentage of tubers showing blackening.
Epicure	⅛-3″	59
Epicure (large)	⅛-3″	89
Arran Pilot	open-3″	50
Home Guard	3-4″	32
Catriona	opening	53
Doon Star	open-¼″	5
Majestic	opening	53
Arran Banner	open-¼″	64
King Edward	open-2″	25
Dunbar Standard ..	open-½″	50
Golden Wonder	open-¼″	53

An experiment was conducted in February, 1950, with ten different varieties, twenty tubers of each variety being dropped from a height of four feet. These tubers had been stored under the same conditions after lifting and were all as far as possible of a similar size. Epicure tubers of a larger size were also included for a comparison of the development of blackening on large and medium-sized tubers. It was found that many of the tubers became cracked on striking the floor and in most of these the tissue around the cracks assumed a greyish colour which, however, never extended beyond the vicinity of the cracks, and such discoloration was ignored. Counts were made, after twenty-two days, of those tubers which developed blackening without showing signs of cracking and these results are given in Table II.

The experiment was repeated in June, 1950, with tubers which had been kept under the same conditions and were from the same stock as before. Sprouting was fairly extensive in all varieties and as all the potatoes were in a relatively flaccid condition, there was no cracking after dropping. Since blackening occurs much

TABLE III.

Development of internal blackening in ten varieties—June, 1950.

Variety.	Total tubers.	Degree of blackening.			
		Severe.	Moderate.	Slight.	Index.
Epicure	36	27	9	0	64
Arran Pilot	10	7	2	0	59
Home Guard	38	32	2	3	65
Catriona	14	9	5	0	59
Doon Star	32	3	3	11	12
Majestic	37	18	13	3	47
Arran Banner	46	17	13	14	37
King Edward	35	25	10	0	62
Dunbar Standard	29	15	12	1	51
Golden Wonder	39	19	20	0	52

more extensively and rapidly later in the storage season, the tubers were assessed after seven days for degree of discoloration, " severe " being over 50 per cent. of the cut surface, " moderate " between 10 per cent. and 50 per cent. and " slight " up to 10 per cent. An index figure was then obtained for each variety by multiplying the number in each class by the average percentage of that class, i.e. severe × 75, moderate × 30, and slight × 5, the total being then divided by the number of tubers used. The results are shown in Table III.

By comparing the relative figures for degree of blackening in Tables II and III, it may be seen that, with a few exceptions, there are no great varietal differences in blackening susceptibility between the varieties used. In the earlier experiment Home Guard and King Edward appeared to be less susceptible and Doon Star very markedly so, while Arran Banner appeared slightly more susceptible. The results for Home Guard correspond with previous observations. Later in the season, however, Home Guard and King Edward gave results similar to most of the other varieties, while Arran Banner showed an unaccountably low figure and Doon Star continued to exhibit a marked degree of resistance. It should be noted that all the varieties, with the exception of Doon Star, were grown in the same field, the potash and phosphate status of which was approximately the same as that of the Doon Star field. The pH in the latter, however, was only 5·5 compared with about 6·5 in the other field, and although it is not known whether or not this would have any effect, the fact should be borne in mind before laying too much emphasis on the resistance of this variety.

It may be observed from Table II that large Epicure tubers developed much more blackening than the smaller tubers, presumably because of the greater momentum at the time of impact.

It might have been expected that those varieties which sprouted more rapidly would be less turgid and therefore more susceptible to blackening. It is obvious from Table II that this is not the case. For example, Home Guard tubers which carried sprouts 3-4 inches long showed much less blackening than those of Arran Banner the sprouts of which were only ¼ inch long. It has been suggested that varietal susceptibility to blackening is related to starch content and thus to the percentage dry matter, but this is not borne out by the above experiments, for example, by comparing Golden Wonder or Doon Star with Arran Banner, which have respectively high and low percentage dry matter (Wager, 1946). Mulder (1949) found variations in rates of blackening in twelve Dutch varieties and concluded that this was due to differences in the tyrosin content and the liability of the tissues to bruise. No tyrosin estimations, however, were carried out on the tubers used in the above experiments.

B. The degree of bruising.

Botjes (1929) found different degrees of discoloration by dropping potatoes from different heights between 30 and 70 cm. Koblet (1947) observed that by desprouting potatoes in July, even with special care, internal blackening was caused. When no special care was taken the number of affected tubers was increased considerably. Desprouting potatoes or perhaps handling them in the desprouting process was found to act in the same way in the present experiments, although the result was not so marked as when the tubers were bruised directly, nor was its effect obvious early in the season. It is probable that in practice the most general means by which tubers are bruised with the resulting production of internal blackening is on the riddle, relatively late in the storage season when the tubers are either graded for size or desprouted. This was carried out experimentally in April with tubers of twelve different varieties which had previously been stored in boxes and in all cases severe blackening resulted. In this connection an interesting case was observed some years ago in which part of a clamp of Gladstone potatoes had been "turned over", i.e. desprouted and reclamped in mid-April. One month later a consignment was riddled out for ware purposes but was returned as being unfit for human consumption a few days later since tubers were black both on cutting and also after cooking. On examination it was seen that the blackening was associated with a slight shrivelling of the skin, in fact it was possible to select affected tubers on this basis. When the portion of the clamp was examined from which the consignment had been riddled, only an occasional affected tuber was discovered. Since then, further cases have been observed, the history of which indicated that the blackening had been brought about in somewhat similar circumstances.

It is probable that slightly affected seed tubers will be able to grow satisfactorily although it would not be wise to plant badly affected tubers. Experimental evidence indicated that emergence of plants from tubers bruised by riddling prior to planting was slightly delayed and slightly reduced, but this may have been due to sprouts having been broken off in the course of riddling.

C. *Other factors.*

As far as is known, no work has been carried out in Great Britain on the association of mineral deficiencies with blackening after bruising. Several Dutch workers however (Botjes, 1927, 1929, de Bruyn, 1929, van der Waal, 1929, and others), have all found that potatoes which were deficient in potash, i.e. had been grown on potash deficient soils, were much more susceptible than normal tubers. Biewenga (1929) found that the potash content of affected tubers was only about half that of normal tubers but that although the potash content was higher at the rose end of the tuber, it was not sufficiently so to account for the greater incidence of discoloration at the heel end. Botjes (1929) claimed that such potash deficient tubers had a diminished turgor and this rendered them more susceptible to shock. Susceptibility was reduced, according to Botjes (1927) and van der Waal (1929), by liberal application of potash to the soil. De Bruyn (1929) associated blackening rather with high specific gravity than with potassium supply. She claimed that dark spots are evident near the stem end of affected tubers and these extend and become more numerous not long after harvest. These spots, the cause of which is unknown, are anatomically like spraing lesions. She considered that the spotting was an important and predisposing factor, and tubers showing this suffered more from blueing than did those without the necrotic spots and that the blueing was influenced by application of nitrogen to the soil rather than by deficiency of potash. Such predisposing necrotic spots have not been observed in the course of the present work. De Bruyn and Koblet (1947) found, too, that increased application of high nitrogen fertilizers also predisposed the tubers to internal blackening. Mulder (1949), in his study of the influence of N, P, K, Mg and Cu on blackening, concluded that lack of potassium had by far the greatest effect in increasing the tyrosin content of potato tubers and that the two factors responsible for the blackening tendency are high tyrosin content and liability of the cells to sustain injury.

Physiological and histological changes involved.

Horne (1913) stated that the refractive index of the affected cells was altered and that protoplasmic movement stopped and he found a greater quantity of solanin in the affected tissues. Merkenschlager (1929) found that the blue or black discoloration, which he observed on cutting or cooking certain tubers, was due to the formation of melanin by the action of tyrosinase upon the tyrosin which is liberated when the cells are killed. For this reaction the presence of oxygen is necessary and Botjes (1927) stated that oxygen is necessary for the discoloration to occur in the potato. McIntosh (1935) also stated that the discoloration was due, as in the case of Blackheart, to the formation of the black pigment melanin and evidence for this has been given by Mulder (1949).

As a further indication that the enzyme tyrosinase is involved in the production of blackening, the surface of washed tuber slices was treated with a solution of *p*. cresol as used by Robertson (1932) which is oxidized by tyrosinase and may thus be used to indicate its presence. A pink coloration was produced first of all in the region of the vascular ring, extending approximately to those areas which are primarily affected by blackening.*

In a microscopic examination of the affected tissue a noticeable feature is the presence of a dark granular inclusion, presumably of melanin, in the cells of the discoloured area (Fig. 3). The loss of turgidity of these cells is usually fairly obvious but this may also be seen in cells in the unbruised tissue, since loss of turgidity is a pre-requisite for the production of discoloration. The blackening commences round the vascular ring and extends both inwards towards the centre of the potato and also outwards into the external phloem-carrying parenchyma, both of which regions contain vascular elements. Blackening is often absent in the true cortex and in the central medulla which contain no vascular tissues. In longitudinal sections the early stages of blackening may occasionally be seen as a streaking of the vascular tissue, very similar in appearance to frost necrosis.

The blackening subsequent to bruising may sometimes be confused with Blackheart. Both are more obvious towards the end of the storage season and both are believed to involve the same biochemical reaction. The former, however, invariably begins round the vascular ring; the latter usually, although not always, in the central medulla.

In more advanced stages the blackened tissue may be bounded by a brownish layer reminiscent of blight infection. Detailed examination of this area shows a certain degree of necrosis as well as traces of suberization of the cell walls. It is noticeable in microtome sections that many of these suberized cells retain the dark granules and starch grains which have been cleared from the tissue on either side (Fig. 4). No sign of a wound cambium was present even in material in a very advanced condition examined after about one month, and this is probably due to the loss of moisture of the tissues which reduces their ability to form wound cork. It confirms Horne's (1913) original observation and further provides a histological difference between the blackening which follows bruising and Blackheart, since a cork cambium is frequently found in the latter case (Fig. 5).

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* Mulder (1949) noted that the tyrosinase activity in the outer tissues is more than twice that in the interior. He also showed that lack of copper decreased the tyrosinase activity in normal tubers and that blackening of tubers deficient in both copper and potassium was slight compared with that where only potassium was deficient.

Method of Prevention.

If the tubers could be treated with such care as to avoid all bruising and damage probably most of the blackening which has been described could be avoided. In the normal course of events this is not possible, but as much care as possible should be exercised in handling the tubers especially after storage. It is normally impracticable to avoid riddling potatoes for either seed or ware but it should be borne in mind that blackening may result if tubers which have become flaccid through sprouting are treated in this way.

Dutch work has shown that tubers grown in soil deficient in potash or to which excess nitrogen has been added are more susceptible to blackening after bruising. It is obvious, therefore, that balanced manuring will play an important part in reducing the amount of affected tubers. It is known also that tubers grown on potash deficient soil tend to turn black after cooking, due to an entirely different reaction without formation of melanin, but it may be that slight bruising even although undetectable previously may accentuate the discoloration subsequent to cooking.

Even apart from manurial deficiencies, any factor which will bring about a reduction in turgidity of the tubers will tend to predispose the tubers to the effects of bruising. Consequently, the relative humidity of the storage atmosphere should not be too low. If sprouting can be kept to a minimum, either by low temperature storage or by the action of sprout inhibitors, the tendency to blackening will also be greatly reduced.

SUMMARY.

1. The internal blackening of potato tubers which follows bruising is described.

2. The tubers must be in a relatively flaccid condition before blackening will occur to any extent, and factors such as temperature, duration and conditions of storage which favour loss of turgidity render the tubers more susceptible to the effects of bruising.

3. Susceptibility is greatest at the heel end and least at the rose end of the tuber.

4. Certain varietal differences were observed but these could not be related to the degree of sprouting of the tubers or starch content of the variety and most of the varieties tested showed similar reactions. Large tubers were more susceptible than small ones.

5. The degree of bruising affects the development of blackening and it is probable that in practice one of the most common causes of blackening is the combined process of desprouting and riddling tubers late in the storage season.

6. Reference is made to Dutch work showing that deficiency of potash and excessive nitrogen applications may also increase the susceptibility of the tubers and that the blackening of the tissue is due to the formation of melanin in the action of tyrosinase upon tyrosin in the bruised cells.

7. Even in advanced stages of blackening no evidence of a cork cambium was observed surrounding the discoloured tissue.

8. Methods of prevention are discussed.

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(*Received* 24/7/50.)

ROYAL HORTICULTURAL SOCIETY

THE JONES-BATEMAN CUP FOR RESEARCH IN FRUIT GROWING.

The Jones-Bateman Cup, presented by Miss L. Jones-Bateman, of Cae Glas, Abergele, in 1920, for the encouragement of fruit production, is offered for award triennially for original research in fruit culture which has added to our knowledge of cultivation, genetics, or other relative matters. The cup is offered for award in 1951.

Candidates should submit accounts of their work by 31st October. The work dealt with should have been mainly carried out by the candidate in the United Kingdom and mostly during the last five years. The Cup will be held for three years by the successful candidate, who must give a bond for its safe return. The holder will be able to compete again on the next or any succeeding occasion. When the cup is relinquished the holder will receive a Hogg Medal which will be specially struck in gold if conditions allow it, otherwise a token gold medal will be substituted. The Assessors will be three (two appointed by The Royal Horticultural Society and one by the National Farmers' Union), and they will report to the Council of the Society upon the originality and comparative potential value to the fruitgrowing industry of the work of the candidates.

The Jones-Bateman Cup has been awarded as follows :

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1945 Dr. W. S. Rogers.
1948 Dr. A. Beryl Beakbane.

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C. V. L. LYCETT, *Secretary.*

REVIEWS

THE HORTICULTURAL NOTEBOOK. By J. C. Newsham (revised by W. E. Shewell-Cooper). Fourth Edition. (The Technical Press, London, 1950, pp. 418. Price 10s. 6d.)

This is a most disappointing book to review for it teems with inaccuracies ancient and modern and is sadly out of date in much of its information. The best one can say is that it will probably be welcomed by many and may be helpful since it contains a useful collection of the facts and figures used in the everyday running of a garden, a nursery or a fruit farm.

H.W.M.

AMERICA'S CROP HERITAGE. By N. Klose. (Iowa State College Press, Ames, 1950, pp. 156, illus. Price $3.50.)

This fascinating sketch of plant introductions into the United States shows clearly the very important part played by such men as Fairchild, Lathrop, Hansen, Carleton, Knapp and the earlier statesmen of the U.S. in increasing the agricultural wealth of the Western hemisphere by introductions from Europe in particular, but also from Asia and other parts of the world. It is certainly news to some of us to read of the very serious attempt to found a tea industry in Southern California in the middle of the nineteenth century, which ended with the closing down of the Experimental Tea Farm in 1887, but not before hundreds of households had learned to grow their own tea. (Do they still do so ?) Similarly coffee was tried but proved even less successful, in Florida, California and Texas. Olives show quite a different picture since the time when the Franciscans set out groves in California three centuries ago. The Deglet Noor date introduced by W. T. Swingle from Algeria in 1900 is now the basic variety of the present date industry in the United States. The tale of the introduction of the Smyrna fig and eventually the establishment of the wasp *Blastophaga psenes*, so essential to its commercial success, makes good reading. Galloway's estimate in 1928 affords a rough idea of the importance of fruit introductions. According to this, the number of fruit crop varieties introduced and inventoried by the Office of Seed and Plant Introduction up to that date were : apples 600, avocadoes 353, blackberries 100, jujubes 225, mangoes 498, nectarines 50, peaches 500, pears 700, oriental persimmons 600, and plums 450. Other introductions included vegetables, sugar cane, tobacco and various cereals. Regional activities are now centralized round 4 primary introduction stations at Pullman, Wash. for the West ; Ames, Iowa for the North Central Region ; Glenn Dale, Md. for the North East ; and Experiment, Ga. for the South. The process of seeking, finding and improving continues and between 1946 and 1948 five exploring expeditions left the U.S. in search of potatoes in Mexico, forage plants and ground nuts in South America, and vegetables, oil plants etc., in Turkey.

In 1949 India was being searched for winter fodder cereals and other plants with potential industrial uses.

Redesigning plants to contain special characters is the plant breeder's job and introductions are his raw materials.

D.A.

ORNAMENTAL CHERRIES. By C. Ingram. (London: Country Life, New York: Charles Scribner's Sons, 1948, pp. 259, illus. 48. Price 30s. net.)

This is the monograph for which all who are acquainted with Collingwood Ingram have long waited. Just to state that it is by the greatest living authority on the most delicately beautiful trees in existence should suffice, but happily it is much more than that. Being written in the first person we are enabled to enjoy a vivid picture of this virile author, in fact many of us hear his enthusiastic and incisive tones as we read. Loving and exulting in cherries the author brooks no " ifs " or " buts " from any laggardly convert and no fool is suffered gladly. When told that cherries are only in bloom a short while he cries " one day, or even one hour ". No, to be of his company we must be worshippers indeed!

The book is divided into three parts. In the first we have the history and folklore of the cherry, then the author's interesting experiences in propagation by seeds, cuttings and grafting, the making of his beautiful garden at Benenden, the growing of dwarfed trees in pots and, sadly but inevitably, the diseases and pests which sully the temple. The second part, larger than both the others put together, contains a revised classification of the deciduous cherries and discusses in detail the wild species and their varieties which are at present known to have been introduced into this country, while the third describes the Japanese Cherries of garden origin now to be found here. The wild species, in their ethereal beauty, attract the author far more than the bolder garden forms. In fact, the popular Kanzan he considers rudely glamorous as it stares at us from almost every other suburban garden. Yet we believe this same Kanzan could be effectively used in informal groups in our public gardens. In fact the author commends the mass planting of cherries and urges some English resort to make a sufficiently grand display, of one variety only, to attract thousands of visitors as has been done in Japan and, more recently, in America also.

Delightful as it is to read, in part provocative and continuously stimulating, this book will be treasured chiefly as the authoritative reference for ornamental cherries and no garden designer will neglect to consult it. Every park superintendent should possess a copy and have it beside him, especially when the " committee " is considering the improvement of the landscape. Nurserymen now have a clear guide through a nomenclature maze which hitherto has caused aches in both head and heart. There are thirty-two full page photographs and eight reproductions of delightful watercolours of wild species by the author, as well as half-a-dozen clear line-drawings.

One might have expected that a book by Cherry Ingram would have received better treatment from the publisher. A subject such as this, handled so delightfully by an author of this calibre, should have been matched by the best in modern book design, and our joy completed. Instead, we have a dull page, a type ill-suited to the paper, and sticky binding. R.J.G.

LILIES OF THE WORLD. By H. D. Woodcock and W. T. Stearn. (London: Country Life, 1950, pp. 450, illus. Price 35s. net.)

Originally this book was intended to form the third edition of *Lilies, Their Culture and Management* of which the second edition was published in 1936. It was found, however, that the extensive progress made since then in every branch of lily lore, scientific and horticultural, had rendered any satisfactory revision impossible, and so an entirely new work has been produced under another title. Thoroughly workmanlike and definite, though by no means pontifical, on matters of cultivation and unquestionably authoritative on the botanical problems of classification and distribution, this book by its very completeness cannot fail to become the standard work on lilies and to remain so for many years.

Lilies have always been regarded as difficult plants, many gardeners, indeed, being afraid to touch them. It is encouraging to learn here that by taking a few simple precautions (in some cases none at all) the difficulties should prove illusory. In many ways lilies respond better to austere conditions than to soft living. Those described in this book, at a rough estimate some 400 species and varieties, many, of course, of garden origin, will grow in any ordinary garden soil in one part of the country or another, though only a supreme optimist would hope to succeed with all lilies in any one locality in Britain or any other land.

Part 1, which is concerned mainly with cultivation, wisely assuming a total ignorance on the part of the reader, opens with a clear exposition of the structure of the lily, amply supported by first-rate drawings of which those illustrating the various types of lily bulb are particularly to be commended. The chapter on soil preferences is brief, for these are few. It contains useful suggestions for circumventing such English climatic vagaries as frosts in May or excessive winter wet and gives a helpful list of twenty-eight species or varieties which are tolerant of lime or even like it. There are also short lists of easy, moderately easy, and difficult outdoor lilies, this last category presenting a challenge which surely no gardener worthy of his salt will fail to take up.

Advice on manuring consists largely of warnings, but the amateur will run no risk if he confines himself to hoof and horn and bone meal and a little sulphate of potash dug in before planting. Oak and beech leaf-mould is the lily food par excellence. The chapter on propagation is of great interest, for few genera possess a more varied means of increase. Seeds, daughter bulbs, scales, bulblets on the lower stems and bulbils in the leaf axils can all be used and the two last can be artificially induced. All these methods are fully described. The work of Miss L. V. Barton of the Boyce Thompson Institute, of the Buckleys in Canada, and of others on methods of stimulation in the case of lily species, characterized by delayed hypogeal germination, is specially noted. There is a chapter on hybridization. In conclusion an account of pests and diseases, in the authors' words, makes dismal reading. However, comfort may be taken from the fact that many are avoidable and at least that no one will have them all at the same time. Fortunately, some of the pests and diseases have not yet left America.

Part 2 takes up the greater part of the book and is concerned chiefly with classification and description, giving very full notes on all the known species and many hybrids. The fine photographic plates of nearly 100 species are an outstanding feature and there are in addition many line drawings. For easy reference the plates are distributed alphabetically throughout the book. The genera Cardiocrinum, Notholirion and Korolkowia are now included. The first-named has recently engulfed the majestic *Lilium giganteum*, a change which gardeners will deplore, and probably ignore, for surely with so unfamiliar and uncouth an appellation this celebrated lily can never seem quite the same to its devotees. The book is well documented throughout and furnished in addition with an extensive bibliography. A handsome volume with nothing shoddy about it, it competes successfully in appearance and solidity with any comparable production of those dim halcyon days known as " pre-war ". As regards treatment of its subject it is in a class apart, and as regards price, if this may be mentioned with seemliness, the kindest thing to say is that it is up to date. G.St.C.F.

COMMERCIAL GLASSHOUSE CROPS. By W. F. Bewley. (London: Country Life, 1950, pp. 508, illus 128. Price 50s. net.)

The twelve chapters of this large volume cover the whole field of glasshouse cropping. No single book has ever before dealt so exhaustively with flowers, fruit and vegetables under glass; it is both literally and figuratively a *magnum opus*. It represents the accumulated knowledge and experience of the author, for over thirty years Director of the Cheshunt Experimental Station. So much work has been carried out on every aspect of commercial growing under glass by this Station that it is understandable that the author draws most of his data to support his arguments from the results obtained there.

The first chapter deals with the planning and building of a glasshouse nursery and gives a wealth of detail on choice of site and location, the construction of standard type timber houses and the more usual methods of heating. There is, unfortunately, no mention of metal or concrete houses and therefore no discussion of their merits and demerits, a matter of very considerable argument at the present time, when there is still difficulty in obtaining adequate supplies of well-seasoned timber. Similarly, the new development of heating by low pressure steam receives little or no discussion, although many growers are now favouring it on grounds of economy of running costs and more accurate temperature control. Chapter II is concerned with the soil, and is a clear and lucid account for the grower of the principles of plant nutrition, soil management, the maintenance of fertility and the use of fertilizers. The value of soil warming by hot-water pipes or by electric cables or low-voltage wires is discussed, with special regard to tomatoes and bulbs.

Chapter III is a complete guide to tomato growing and, as might be expected, is by far the longest chapter in the book. Probably there has been more research on the tomato than on all other glasshouse crops put together. The notes on the history of the tomato and the classification of the genus *Lycopersicon* are interesting, and the descriptions of the fourteen groups into which the commercial varieties of tomato fall are useful, though more detail, especially of leaf characters, would be helpful in the identification and recognition of varieties. Attention is drawn to the need for careful selection in the maintenance of varieties, well illustrated by the Cheshunt strain of Potentate, a variety which, if unselected, produces many large irregular fruits of poor flavour, though its heavy-cropping qualities make it perhaps the most widely-grown of all. A valuable section deals with the relation between light, temperature and nutrition and the growth and cropping of the plant, a subject which should be carefully studied by all growers. No mention, however, is made of the work of Hoagland in California on the inhibitory effect of high night temperatures on the initiation of flowers, though the more empirical results obtained at Cheshunt bear out his fundamental studies.

The difficult art of watering tomatoes, which the author so rightly stresses as the all-important factor in management, receives the detailed consideration to be expected from Dr. Bewley, the originator of winter flooding and overhead damping. From a wealth of material, one might pick out one section for comment; a detailed account of the blemishes which reduce the market value of fruit includes no mention of " blossom-end rot ", a most surprising omission in view of its connection with the water factor and the author's own work on this disorder.

The growing of tomatoes in low houses such as cucumber houses by methods evolved at Cheshunt during the war, is described in detail and a section is devoted to tomatoes under lights and cloches and in the open.

The growing of cucumbers, melons, lettuce and general vegetable crops is described in Chapter IV. In the section on lettuce, more discussion of the merits of the different varieties and their characteristics would be valuable ; the utmost care in handling the young seedlings in planting-out to avoid *Botrytis* infection is not stressed. It has been demonstrated beyond question that damage to the cotyledons at this stage is fatal.

Chapter V deals with fruit crops, the description being confined to grapes, peaches, figs and strawberries. While the idea of allowing peach trees to fruit from the maiden year, as practised by some growers, is discussed, no mention is made of the method of growing these trees on frames set transversely across the house, a method which allows of considerably larger crops, though less use can be made of the house in winter.

Chapters VI, VII and VIII are concerned with cut flowers, bulbs and pot plants respectively. To many growers the first of these will be very valuable, as so little reliable information is available on the growing of certain flower crops, especially orchids, roses, gardenias and sweet peas. In the chapter on bulbs an account is given of the Dutch work on the " preparation " of bulbs for forcing ; though three references are cited, one of these is in Dutch and another in a little-known journal, while no reference is made to the excellent articles by Miss Purvis in *Scientific Horticulture*, Vols. 5 and 6. A valuable account of the growing of pot plants for market is given in Chapter VIII though rather less than justice is done to the work of Lawrence at the John Innes Horticultural Institution, either here or elsewhere.

A chapter on vegetables in the open seems a rather curious addition, but is justified on the grounds that some glasshouse growers wish to grow a few vegetables on outside land.

Disease and pest control is dealt with in Chapter X ; this is not intended to be a guide to the control of all troubles of glasshouse crops, but is concerned mainly with general hygiene, soil sterilization and the uses of insecticides and fungicides. As the originator of steam sterilization, Dr. Bewley justifiably devotes considerable attention to this important subject, which is described with a wealth of detail.

An interesting historical survey of the glasshouse industry traces its growth from its beginning less than a hundred years ago and discusses the impact of two wars and of intensive research in the last half-century upon the expansion of the industry.

The final chapter is concerned with some recent applications of science to glasshouse crops and deals with such diverse subjects as soilless cultivation, irradiation of growing crops, photoperiodism and the uses of growth-regulating substances. In the first-mentioned subject one might venture to suggest that the recommendations as to nutrient solutions could be simplified by discussing the requirements of plants for the nutrient elements in terms of " parts per million " and providing a table of factors for the commoner salts, relating their composition to the amounts required to provide a given concentration. This makes variation in the concentrations of the principal nutrients according to the stage of growth of the plants and to the weather much easier.

R.H.S.

SPLIT LEAF BLOTCH DISEASE OF THE HOP *(HUMULUS LUPULUS)*

By W. G. KEYWORTH
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SALMON and Ware (1928) referred to a disorder of hops seen by them in 1926 as Split Leaf, because the laminae of affected leaves split into irregularly shaped holes. Later (1930) the same authors stated that no severe damage was caused by Split Leaf on the Fuggle variety, but that one or two Wye seedling varieties were greatly stunted by the disease. Fuggle scions grafted on affected seedling plants grew vigorously and showed no symptoms. It was noted that the symptoms of Split Leaf were often present on plants affected with Nettlehead disease.

In 1933, Salmon and Ware (1934) noted a condition which they described as an aggravated form of Split Leaf. It was characterized by the presence, on affected leaves, of yellowish " oily-looking " blotches in which the leaf tissue eventually died and turned brown, or split. This condition was called Split Leaf Blotch. Several outbreaks were reported during 1933 from hop fields in both the south-eastern and west-midland hop growing areas. In an account of observations made near Faversham in Kent these authors (1935) noted that plants showing symptoms in 1933 remained affected in 1934. In addition there was some evidence of spread to new plants.

Observations were later made on the disease by various workers and Keyworth and Davies (1940) emphasized its prevalence in the west-midland area. No further experimental work was done, however, and the exact nature of the disease remained obscure.

Wilkinson (1947) made preliminary experiments which gave evidence that Split Leaf Blotch was graft transmissible and therefore presumably of virus origin. He also noted the prevalence of the disease in Worcestershire and Herefordshire, and concluded that no Fuggle plantation was completely free from it and that, in many, up to 75 per cent. of the plants were affected. He described the symptoms and correlated a "spidery hop" symptom, seen early in the season, with the leaf symptoms appearing from June onwards.

The first part of the present investigation was made in collaboration with Wilkinson and, in 1946, a joint experiment was started to follow up the earlier grafting work. Results from this experiment are described below.

SYMPTOMS.

As reported by Wilkinson (*loc. cit.*) the first symptom is the " spidery " appearance of affected plants early in the season. Normal hop plants produce up to 100 leafy stems from the pruned rootstock in March and April, giving a very bushy plant. From six to eight of these stems are selected for training and cropping. Plants affected by Split Leaf Blotch produce only a few stems, some of which are small and stunted ; the remainder are of normal length but bear only a few small leaves. Such stems are often a dark green or reddish colour. Affected plants usually occur in small groups or rows and can easily be distinguished from their normal neighbours. After the stems are trained up the strings, however, this distinction is lost. Photographs of a normal and of a " spidery " plant are shown in Fig. 1.

Later in the season, leaf symptoms appear on the affected plants. On the young expanding leaves irregular areas of the lamina are of a light green colour. These areas may increase in size and gradually lose all green colour, turning first pale yellow and then becoming transparent and oily in appearance. Uneven growth of the leaf causes considerable distortion and eventually the tissue in the affected patches may die and fall away, leaving irregular holes and splits (Fig. 2). Great variation may occur in the severity of the symptoms on different plants. Thus on some plants only one small affected area may be noted on one of the leaves, whereas on others very many of the leaves may be badly affected. There is often a considerable reduction in the vigour of the plants showing severe leaf symptoms.

The readiness with which symptoms can be seen often varies during the growing season and also from year to year. On experimental plants kept under observation at East Malling during 1947 symptoms appeared in early July on the young expanding leaves. On all plants these leaves continued to show symptoms subsequently, but, whereas on some plants more young leaves were affected, on others the new leaves produced in early August appeared normal. Observations by Beard* on the clonal commercial hops and certain Wye seedlings grown at East Malling indicate that symptom expression varied from year to year in the plantations in question.

He reports that all the plants of the Tutsham clone, planted in 1934 and grubbed in 1942, showed severe symptoms in 1937 and 1941, but not in other years. Of the 96 plants of the Fuggle L clone, 32 showed moderately severe symptoms in 1941 and 20 were affected in 1947. Symptoms were also seen on one plant of the N clone in 1941. It is noteworthy that two other disorders of unknown cause were particularly obvious in 1941, e.g. " Ivy Leaf " on 26 Wye seedlings bred from a Manitoba hop and Mosaic on the Wye seedlings P13, OO63 and OP21 (which are normally symptomless carriers of this disease). These records suggest that in 1941 the symptom expression of Split Leaf Blotch and these other disorders was influenced by certain unknown factors peculiar to that season.

On the other hand, all the plants of the Wye seedling variety DD47 were severely affected by Split Leaf Blotch in every year of their growth (from 1931 to 1942). In many commercial fields also, plants of the Fuggle variety have shown Split Leaf Blotch consistently for several years.

In the course of field observations, great variations have been noted between different fields in the incidence and severity of the symptoms of Split Leaf Blotch. Whereas in some cases very many plants were severely stunted by the disease, in others only slight symptoms were seen. It has also been observed that Fuggle plants affected with Nettlehead disease usually also show symptoms of Split Leaf Blotch. These symptoms have often been seen on plants known to be affected with Nettlehead, even when the typical Nettlehead symptoms of leaf curl and inability of the stems to climb were not evident because of symptom masking.

GRAFTING EXPERIMENTS.

These were started in 1946 and were designed to supplement the preliminary experiment made by Wilkinson in 1944-5. Cuttings were collected during the autumn of 1945 from plants of the Fuggle variety which had shown symptoms of Split Leaf Blotch during the growing season. These plants were selected from two fields, one near Tonbridge in Kent and the other near Hereford. The cuttings were divided into two lots, one for use at East Malling and the other for use at the Long Ashton Research Station, and were planted in 4½ in. pots in the glasshouse. It was not possible, however, to continue the experiment at Long Ashton after 1946 and thus records are available only for those plants grown at East Malling.

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* Private communication.

The method of grafting, by inarching a stem from each infector plant to one from a healthy (indicator) plant was the same as that described by Keyworth and Davies (1947). 143 grafts were made by this method at varying dates from 9th April to 16th June. 122 were successful, the failures being mainly caused by the death of the bine from the Split Leaf Blotch infector plant. Ungrafted controls were grown beside the grafted plants during the 1946 season, the number of such controls being approximately the same as the number of grafted plants. The indicator plants and controls were grown from cuttings from the East Malling N clone of the Fuggle variety. These plants were not entirely free from Split Leaf Blotch as a few of the parent plants had occasionally shown mild symptoms of the disease. A record was therefore made on 24th June, 1946, of the symptoms on most of the control, infector, and grafted indicator plants, the symptoms being graded as 1, 2, or 3, in ascending order of severity (see Table I).

The symptom categories were determined as follows :—

1. Occasional blotches on leaves.
2. Some leaves with extensive blotches.
3. Several leaves much blotched and distorted—growth of stem much reduced.

TABLE I.

Numbers of plants showing Split Leaf Blotch on 24th June, 1946.

Plant category.	Source of cuttings.	No. of plants recorded.	No. of plants in symptom categories.			Total %
			1	2	3	
Infector	Kent	31	9	7	3	72
	Hereford	85	25	22	18	
Indicator ..	Fuggle N clone	127	4	—	—	3
Control	Fuggle N clone	128	14	—	—	11

It will be noted that whereas only 7 per cent. of the control and indicator plants together showed mild symptoms of Split Leaf Blotch in June, 1946, 72 per cent. of the infector plants showed symptoms varying from mild to severe. The proportion of Hereford plants showing severe symptoms was somewhat greater than the proportion of those from Kent.

No further symptoms were seen on the indicator plants during 1946. All the control plants and those indicator and infector plants which had been successfully grafted were planted out of doors for observation during 1947. There was a considerable mortality after planting, probably partly due to the very dry weather during the spring, but whereas the controls and indicator plants showed a loss of 17 per cent. and 21 per cent. respectively, 32 per cent. of the infector plants died. This, and the death of some of the infector plants in the glasshouse in 1946, suggest that the diseased plants had a lower survival rate than normal ones. The same effect was noted by Wilkinson (*loc. cit.*).

Symptoms were noted on the surviving plants during 1947. A few showed symptoms on 2nd July, and on 12th July many more were recorded. A further record was made on 2nd August, but by this date severe red spider damage tended to obscure the symptoms of Split Leaf Blotch. The numbers of plants showing symptoms are given in Table II.

TABLE II.

Numbers of infector, indicator and control plants showing symptoms of Split Leaf Blotch during 1947.

Type of plant.		No.	No. with symptoms† (in categories).			Total %
			1	2	3	
Infector	From Kent	27	8	7	2	67
	From Hereford	55	12	22	4	
Indicator	Grafted to Kent infectors	35*	14	11	2	63
	Grafted to Hereford infectors	61*	15	19	0	
Control	—	100	9	—	—	9

* The fact that a larger number of indicator plants was available than infectors to which they had been grafted was due to the mortality of the latter.

† For description of symptom categories see p. 165.

A comparison of Tables I and II shows that the proportion of control plants with symptoms was approximately the same in both 1946 (11 per cent.) and 1947 (9 per cent.). None of the control plants showed severe (Group 2 or 3) symptoms in either year. The proportion of infector plants with symptoms was also approximately the same in both years (72 per cent. in 1946 and 67 per cent. in 1947) but the symptoms were of the severe type on many of the plants.

The proportion of indicator plants with symptoms increased from 3 per cent. in 1946 to 63 per cent. in 1947 when many of the plants showed symptoms of the severe (Group 2) type. It is thus apparent that the change in the symptom status of the indicator plants had been caused by grafting them to the infector plants. From this it may be concluded that the more severe symptoms induced in the indicator plants were caused by virus infection, the virus having passed from the infector plants.

SPLIT LEAF BLOTCH DISEASE OF THE HOP
(*Humulus lupulus*). BY W. G. KEYWORTH.

PLATE I.

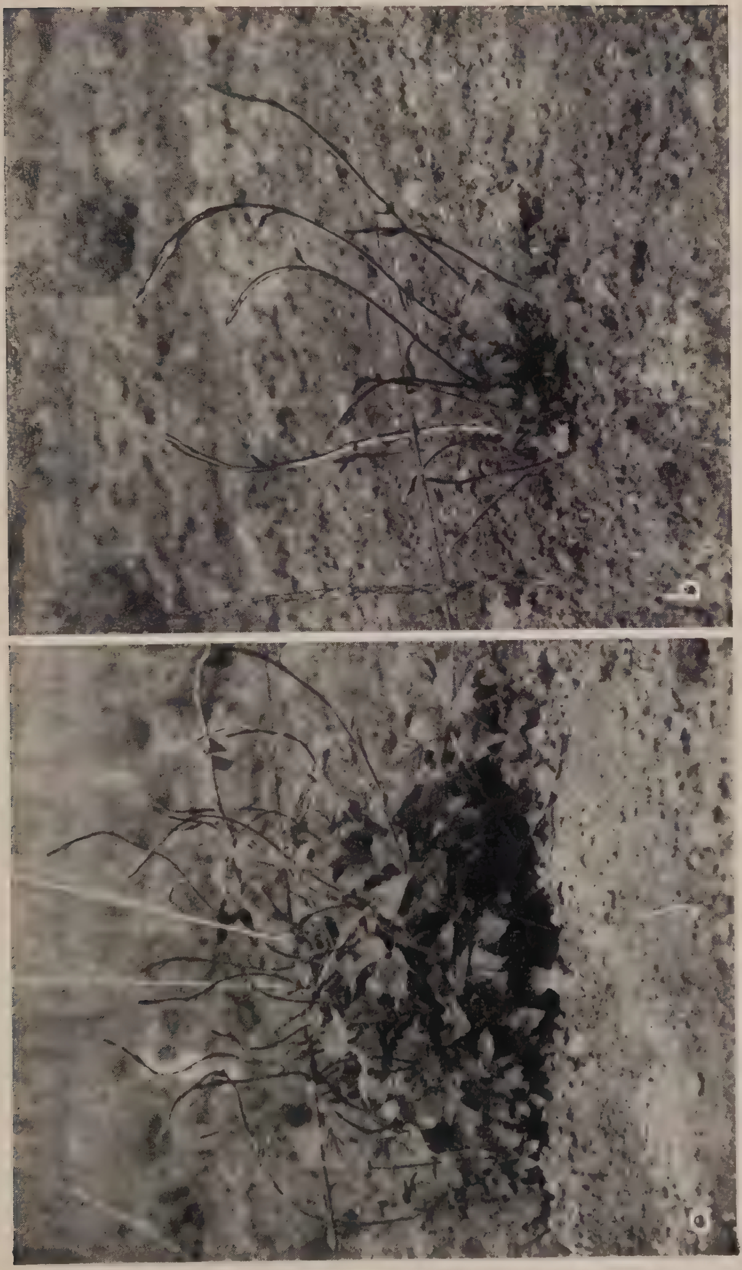

FIG. 1.

"Spidery Stock" symptom—seen in early May.

(*a*)—Normal plant; note bushy appearance. (*b*)—"Spidery" plant: note few stems.

PLATE II.

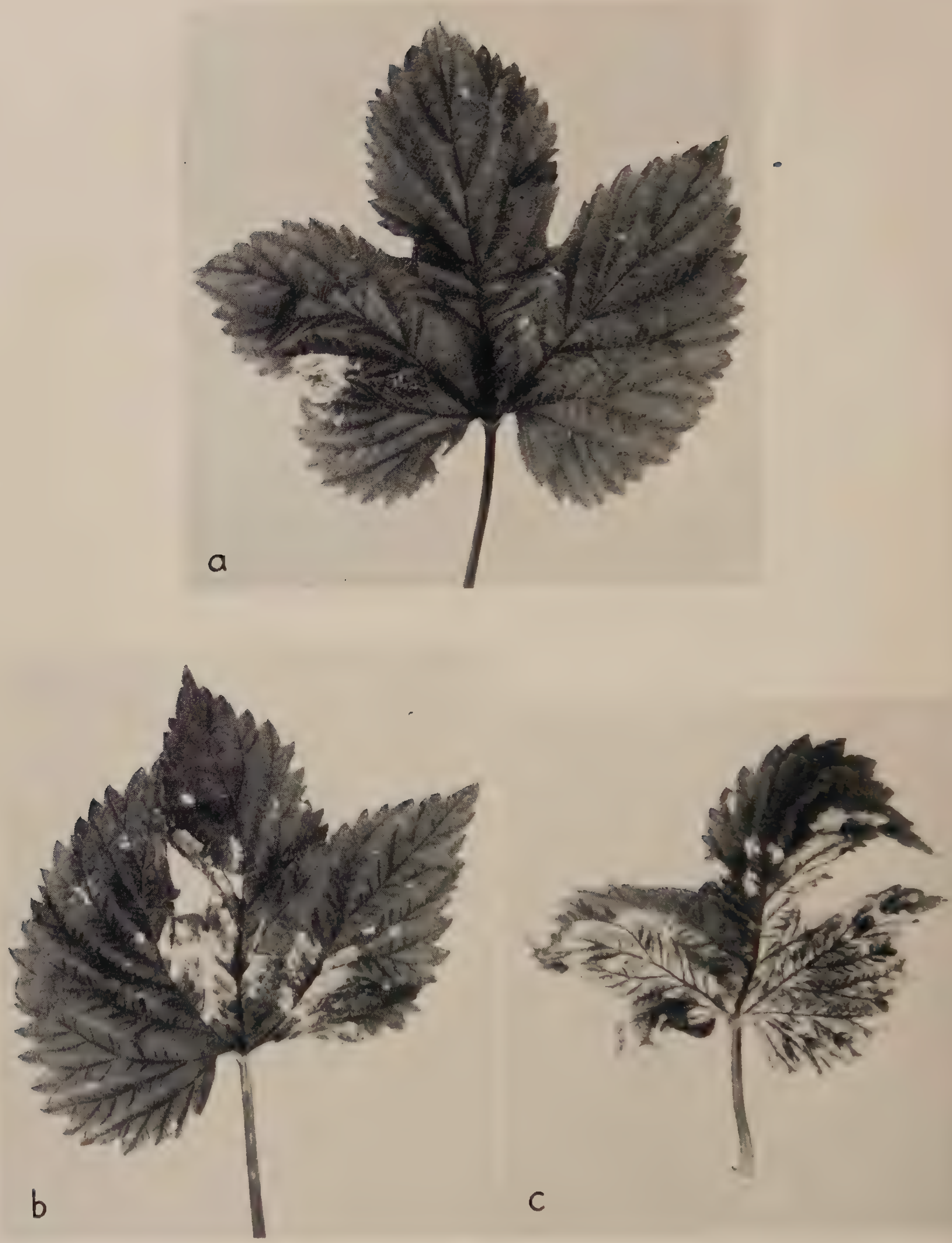

FIG. 2.

Split Leaf Blotch—leaf symptoms seen in July.

(*a*)—Category 1. Small blotch on edge of leaf.

(*b*) and (*c*)—Category 2. Extensive blotches with severe leaf distortion.

NOTE.—Category 3 leaf symptoms as (*b*) and (*c*) but growth of the whole stem is stunted also.

The symptom records given in Tables I and II suggest that some 30 per cent. of the infector plants were not diseased although they were taken from infected parent plants. This is due to the fact that many of the infector plants showing symptoms in 1946 did not do so in 1947 and *vice versa.* Of the 75 plants which were recorded in both seasons, only four failed to show symptoms in either year, and it is thus apparent that most or all of them were diseased. The fact, therefore, that only 67 per cent. of them showed symptoms in 1947 suggests that the proportion of infected indicator plants may also have been greater than the 63 per cent. recorded as showing symptoms in 1947.

As already noted, plants affected with Nettlehead often show symptoms of Split Leaf Blotch even when the Nettlehead is masked. For this reason particular care was taken in the present experiment to ensure that none of the plants was Nettlehead-infected. The source-plants of the infector stock showed no symptoms of the disease at any time during the year before the cuttings were collected. No plant in the experiment showed symptoms of Nettlehead either in 1946 or 1947. About half of the infector and indicator plants were retained for observation in 1948. No Nettlehead symptoms were seen on any of them. Previous experience shows it to be unlikely that Nettlehead would have remained masked for the whole time that the plants were under experiment and it may thus be concluded that the plants were not infected with this disease.

DISCUSSION.

The grafting experiments show that, in the absence of other ascertainable cause, the more severe symptoms of the disease known as Split Leaf Blotch are caused by virus infection. It was not possible in the experiments to obtain conclusive evidence on the transmissibility of the milder symptoms included in the general symptom picture of the disease, because the indicator plants included initially a proportion of plants showing mild symptoms. It should be noted, however, that none of the control plants (from the same stock as the indicators) showed severe symptoms in either season. This suggests that the mild symptoms may have had a different cause from that responsible for the severe symptoms. This is supported by the observations already noted on the differences in symptom expression in different plantations. The evidence on this matter is inconclusive, however, and the problem requires further study.

The relationship of Split Leaf Blotch and Nettlehead disease also requires further investigation. The present experiments indicate that viruses exist which can cause Split Leaf Blotch symptoms on plants free from Nettlehead disease. It is not clear, however, from the present information on Nettlehead (Keyworth and Davies, 1946) whether it occurs independently of infection with Split Leaf Blotch.

Mention should also be made of the apparently remarkable increase in the incidence of Split Leaf Blotch since it was first recorded in 1933. It is, of course, possible that the disease had existed unnoticed for many years before that date. The discovery and study of Split Leaf in 1926 renders this less likely, however, since it is to be expected that Split Leaf Blotch would have been recorded then if it was very widespread. Split Leaf is not now recorded in hops, probably because of confusion with the more severe Split Leaf Blotch, and the relation between the two diseases thus remains unresolved.

SUMMARY.

In this account of the hop disease Split Leaf Blotch, the symptoms are described and observations on its incidence and severity are summarized. Experimental evidence to show that the disease can be transmitted by grafting is presented. It is thus presumably of virus origin.

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(*Received* 20/7/50.)

METABOLISM OF MALIC ACID IN APPLES

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THIS study of the metabolism of malic acid in apples is based mainly upon experiments which were carried out during the course of several years and for which Bramley's Seedling and Worcester Pearmain apples were used. The data and the methods of analysis have already been published in full in this Journal (Griffiths and Potter, 1950) and are not included here.

The observations made in these experiments were on the total titratable acidity of apples which were removed from the tree at different stages of development and which were stored at different constant temperatures. The results were expressed in terms of malic acid. In the case of one of the experiments (Experiment V, Bramley's Seedling apples, 1934) the concentration of hydrogen ion in the juice of the apples during storage was investigated by Kidd and Hanes (1937). From the values of pH and titratable acid the approximate concentration of cations present was deduced, using the data of Haynes and Brown (1928) on the pH of mixtures of malic acid and potassium malate. The values for the initial samples indicated the presence of 0·027N alkali, and it was found that there was no significant change during storage, the value fluctuating between 0·024N and 0·029N. Accordingly, the changes in the acidity of stored apples are explicable on the basis of changes in the amount of free malic acid in the presence of a small amount of a monobasic salt which remains constant in concentration.

Dr. Isherwood (personal communication) has shown in this laboratory that in Bramley's Seedling apples, gathered on the 26th June, malic acid accounted for at least 98 per cent. of the total titratable acid present. Various authors (Nelson, 1927 ; Franzen and Helwert, 1923) have recorded citric acid in apples varying in amount from nil to 25 per cent. of the total titratable acid present. Recently Turner (1949), working in Australia with mature Granny Smith apples stored at 0°C., reported that these apples contained citric acid amounting to about one-fifth of the total acid, and that, in contrast to malic acid, the citric acid increased during storage at 0°C.

The present data were in the first place examined in the form of quantity/time plottings. These plottings showed that at all temperatures of storage there was often no loss of acid during an initial period after the gathering of the fruit, a period which at 10° C. may last for as long as three weeks. This initial period during which there was no loss and possibly even a slight rise in acid content, is termed here the delay period. In nitrogen (containing not more than 0·5 per cent. oxygen) the delay period did not occur. Fidler (1935) had previously observed that the rate of acid loss in apples was the same in nitrogen as in air. Following the delay period, a steady decrease in the acid content of the fruit took place, the quantity/time relationship of which is of the logarithmic form previously reported by Haynes (1925).

Critical examination has failed to reveal any evidence that the constant of the logarithmic quantity/time curve for acid loss changes when the respiratory activity increases at the climacteric. It thus appears that the rate of metabolism of malic acid is unaffected by the factors which cause the rise in the rate of CO_2-production at the climacteric.

Data summarising the respiratory activity/time curves are given in Table I.

TABLE I.

Respiratory activity of Bramley's Seedling and Worcester Pearmain apples at different temperatures.

Experiment.	Variety and date of gathering.	Temperature.		CO_2-production (c.c. per 10 K.H.).		
					Climacteric.	
		(°C.)	(°F.)	Initial value.	Minimum value and date.	Maximum value and date.
	Bramley's Seedling					
1.	28.9.28	1	33·8	19·7	18·8 (11.10.28)	—
	,,	3	37·4	22·7	22·6 (2.10.28)	32·0 (20.1.29
	,,	5	41	30·0	28·3 (6.10.28)	38·1 (15.11.28)
	,,	10	50	47·6	42·4 (4.10.28)	66·8 (21.10.28)
	,,	15	59	74·0	60·0 (2.10.28)	102·0 (14.10.28)
	,,	22·5	72·5	114·0	88·0 (1.10.28)	183·0 (9.10.28)
2.	31.8.31	10	50	62·0	42·5 (17.9.31)	59·0 (29.10.31)
	1.10.31	10	50	47·0	38·0 (12.10.31)	60·0 (27.10.31)
4.	23.6.33	1	33·8	55·0	—	—
	,,	3	37·4	80·0	37·0 (18.9.33)	53·0 (17.12.33)
	,,	10	50	145·0	48·0 (2.8.33)	64·0 (15.11.33)
	,,	18	64·4	250·0	33·0 (15.8.33)	94·0 (21.9.33)
5.	10.7.34	3	37·4	60·0	27·8 (30.8.34)	36·0 (17.12.34)
	,,	10	50	77·0	34·0 (4.9.34)	52·7 (14.10.34)
	,,	15	59	124·5	50·7 (31.8.34)	61·2 (15.9.34)
	,,	20	68	160·0	66·0 (13.8.34)	115·8 (21.8.34)
7.	24.9.37	1	33·8	30·0	22·0 (27.10.37)	26·0 (22.1.38)
	,,	5	41·0	37·0	24·0 (18.10.37)	47·0 (1.12.37)
	,,	10	50	57·0	40·0 (16.10.37)	62·0 (3.11.37)
	,,	15	59	66·0	55·0 (1.10.37)	117·0 (21.10.37)
8.	24.7.39	10	50	78·0	37·0 (2.10.39)	58·0 (21.10.39)
	Worcester Pearmain					
3.	3.9.31	1	33·8		25·0	29·0 (30.10.31)
	,,	10	50		60·0	88·0 (16.9.31)
6.	18.7.35	10	50	110·0	81·0 (1.8.35)	128·0 (14.8.35)
	29.7.35	10	50	100·0	74·0 (5.8.35)	121·0 (19.8.35)
	15.8.35	1	33·8		33·0	—
	,,	10	50		75·0	119·0 (31.8.35)
	2.9.35	10	50		72·0	122·0 (11.9.35)

FORMAL PROCEDURE IN ANALYSING THE DATA.

Plottings of the logarithms of the values for acidity calculated as malic acid (gm. per cent., original fresh weight) were made against time. The first observation used was the first that fell below the lowest value recorded for the replicate initial samples. Values obtained from samples at the end of the storage life, which showed wastage due to rots or to low-temperature breakdown, were omitted. Straight lines of best fit, assuming the errors to be in the acid and not in the time variable, were then calculated for the plottings of the logarithms of the acid values against time. The scatter of divergence of the observed values from those thus calculated was generally of the same order as the scatter observed in replicate samples. The delay period is, then, formally defined as the period from the time of gathering and bringing into store to the point where the straight line of best fit cuts a line drawn parallel with the time axis through the mean of the initial samples. An example of this procedure is shown in Fig. 1. Table II records the durations, thus obtained, of the delay periods in the experiments for which the data have been published in a previous number of this Journal (Griffiths and Potter, 1950). The constant a in the logarithmic equation $\log c = b - at$ (in which c = acid content as percentage of fresh weight, and t = time), defines the rate of the disappearance of acid independently of any effect of concentration. Table III sets out values obtained for the rate constant in these experiments, and in Figs. 2 and 3 these values are shown related respectively to maturity at the time of gathering and to temperature of storage. Fig. 4 shows the quantity/time plottings of acid loss in Bramley's Seedling apples gathered on 12th July, 1934 (Experiment V).

TABLE II.

Duration (days) of initial "delay" period after gathering, before logarithmic decline in acidity begins.

Experiment.	Mean of initial samples. (% acid).	"Delay" period in air.							10°C.		
		1° C.	3° C.	5° C.	10° C.	15° C.	20° C.	22·5° C.	N_2	O_2	Gas mixture.*
Bramley's Seedling											
IV. 23.6.33	2·49	—	19	—	17	—	—	—			
V. 10.7.34	2·18	35	21	—	10	5	6	—	0	12	17
VIII. 24.7.39	1·94	—	—	—	17	—	—	—			
II. 31.8.31	1·66	—	—	—	17	—	—	—			
VII. 24.9.37	1·70	13	—	16	16	0	—	—			
I. 28.9.28	1·31	—	20	13	11	6	—	4			
II. 1.10.31	1·48	—	—	—	3	—	—	—			
I. 24.10.28	1·23	—	15	—	—	—	—	—			
Worcester Pearmain											
VI. 18.7.35	1·10	—	—	—	8	—	—	—			
29.7.35	0·97	—	—	—	3	—	—	—			
15.8.35	0·75	3	—	—	0	—	—	—			
2.9.35	0·61	—	—	—	0	—	—	—			
III. 3.9.31	0·56	9	—	—	0	—	—	—			

* 20 per cent. O_2, 10 per cent. CO_2, and 70 per cent. N_2.

In Table IV are given the delay periods and the values for the rate constant obtained from other sets of data in the literature, namely those recorded by Archbold (1932), those by Onslow, Kidd and West (1932 and 1933) for Bramley's Seedling and Worcester Pearmain apples stored at 1° C. and certain sets of unpublished data of our own. These unpublished data were obtained in 1929 from experiments in which the storage behaviour of Bramley's Seedling apples, from trees which had received different fertilizer treatments, was compared.

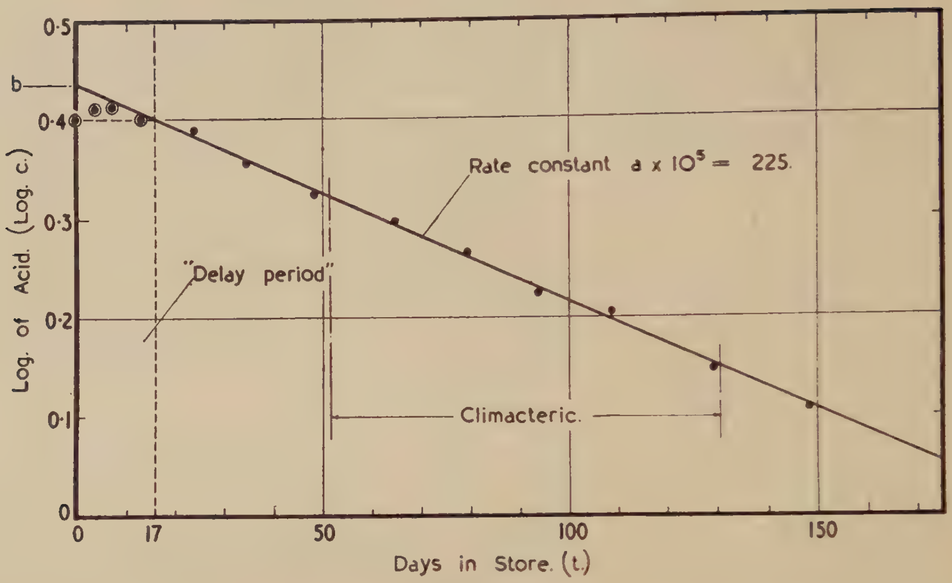

FIG. 1.

Illustrating graphical method of analysing the data (see text), and demonstrating the absence of any effect of the climacteric rise in respiratory activity upon the rate of acid loss. Bramley's Seedling apples stored at 10° C. Experiment IV, 1933. ⊙ Excluded from calculations. • Included in calculations. Between the two vertical lines there was an increase of 34 per cent. in the rate of CO_2-production—the climacteric rise in respiratory activity.

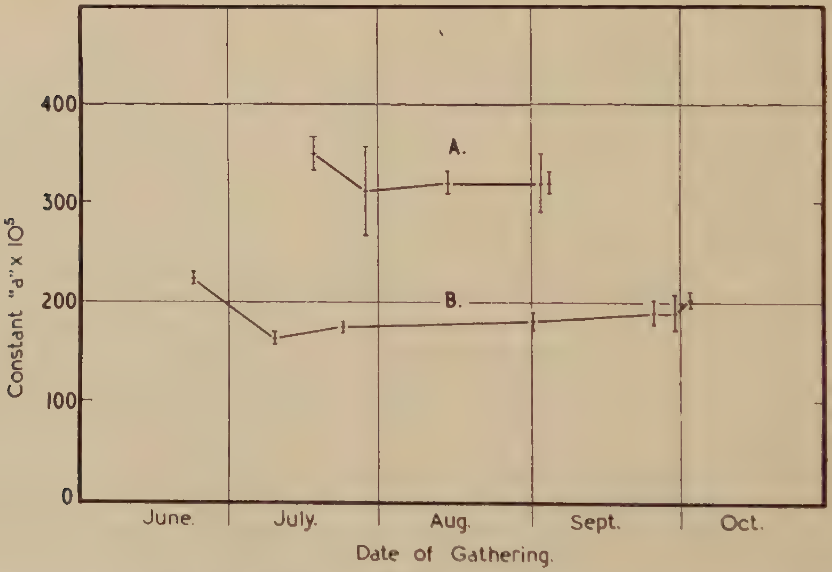

FIG. 2.

Effect of maturity on the rate constant of malic acid loss after gathering (*a*) Worcester Pearmain and (*b*) Bramley's Seedling apples stored at 10° C. Standard errors indicated by vertical lines through the points.

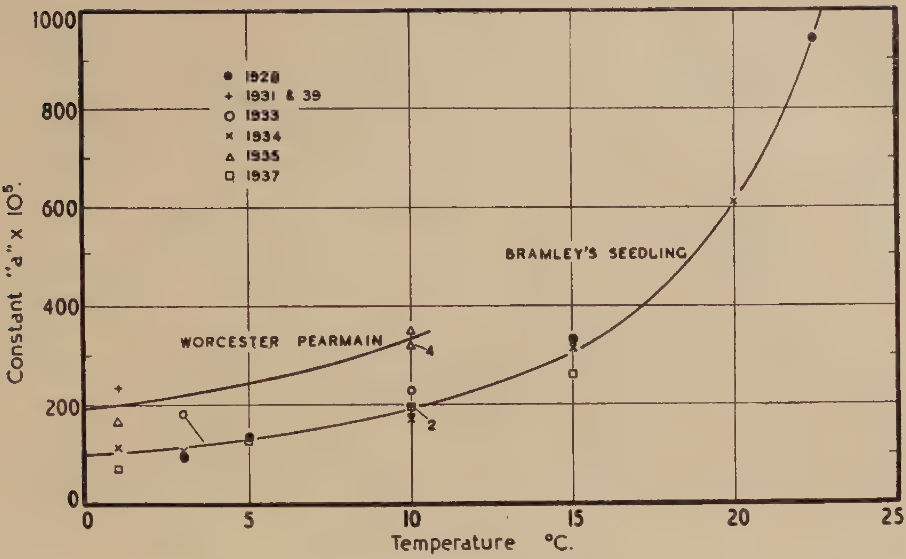

FIG. 3.

Effect of temperature on the rate constant of malic acid loss after gathering in Bramley's Seedling and Worcester Pearmain apples.

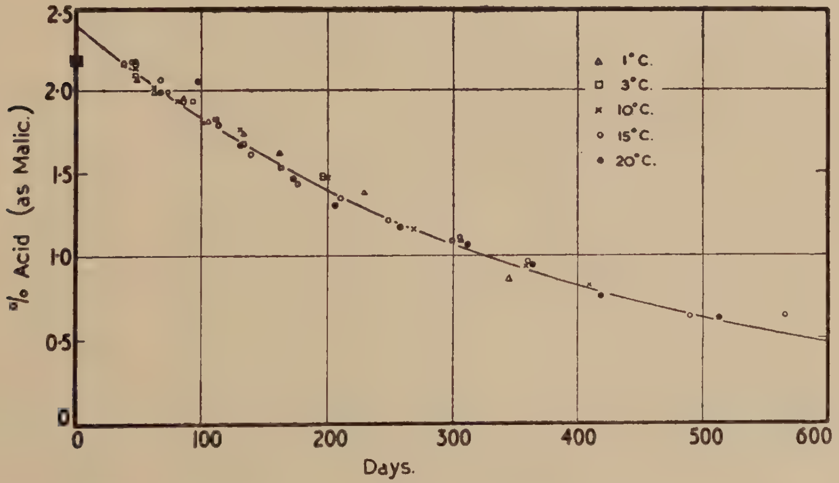

FIG. 4.

Quantity/time plottings of acid (as malic) in Bramley's Seedling apples, gathered on 10th July (av. weight 30 gm.) and stored at 1°, 3°, 10°, 15° and 20° C. Experiment V, 1934. The time scale shown is that of the observations made on fruit stored at 1° C. For fruit stored at other temperatures the time scale is converted to that of the fruit stored at 1° C. by using the ratios of the rate constants, starting from the end of the delay period in each case. The curve shown is the calculated line of best fit for the observations at 1° C. The points during the delay period are omitted. Reference to Experiment V, 1934, in the earlier paper (Griffiths and Potter, 1950) will show that in this particular experiment points were obtained at very close intervals during the "delay" period. All ten initial observations fall within the range indicated on the vertical axis at zero time.

TABLE III.

Constants $a \times 10^5$ *of the equation* $\log c = b - at$, *with their standard errors.*

Experiment.	Storage in air at—							Storage at 10° C. in—		
	1° C.	3° C.	5° C.	10° C.	15° C.	20° C.	22·5° C.	N_2	O_2	Gas mixture.†
Bramley's Seedling.										
IV. 23.6.33		181 ±6·5		225 ±3·8						
V. 10.7.34	116 ±6·2	104 ±2·7		165 ±3·5	308 ±27·3	611 ±20·4		158 ±5·6	157 ±4·1	186 ±5·8
VIII. 24.7.39				176 ±4·1						
II. 31.8.31				178 ±7·7						
VII. 24.9.37	73 ±7·3		125 ±11·6	192 ±10·8	256 ±36·7					
I. 28.9.28	*	97 ±6·5	123 ±6·8	193 ±15·7	324 ±55·1		942 ±21·2			
II. 1.10.31				196 ±5·9						
I. 24.10.28			132 ±11·8							
Average	94	127	127	189	296	611	942	158	157	186
Worcester Pearmain.										
VI. 18.7.35				351 ±16·8						
29.7.35				317 ±44·0						
15.8.35	164 ±6·7			318 ± 9·3						
2.9.35				319 ±30·2						
III. 3.9.31	237 ±46·9			320 ±11·4						
Average	201			325						

* Value omitted because of low-temperature breakdown.

† 20 per cent. O_2, 10 per cent. CO_2, 70 per cent. N_2.

TABLE IV.

Constants $a \times 10^5$ *of the equation* $\log c = b - at$, *with their standard errors; and the duration of initial " delay " period after gathering.*

Experiment.	Date of gathering.	" Rate constant."		Delay period (days) at :	
		1° C.	10° C.	1° C.	10° C.
Bramley's Seedling					
Archbold (1932)	10.9.29	86 ±4·1		0	
,, ,, ...	26.9.29	89 ±5·7		22	
,, ,, ...	22.10.29	73 ±6·5		0	
Onslow, Kidd and West (1932)	1.10.26	105 ±15·9		20	
,, ,, ,, ...	8.10.26	82 ±12·7		37	
,, ,, ,, ...	8.10.26	89 ±20·2		17	
,, ,, ,, ...	8.10.26	95 ±17·4		49	
,, ,, ,, ...	14.10.26	122 ±7·1		37	
,, ,, ,, ...	18.10.26	122 ±18·3		16	
,, ,, (1933) " Ringed " trees	21.9.31	97 ±11·6		7	
,, ,, ,, " Unringed " trees	21.9.31	87 ±8·1		6	
,, ,, (Unpublished) :					
Treated with potash	22.9.29		128 ±11·2		0
Untreated ,, ,,	22.9.29		148 ±10·9		0
Treated ,, ,,	12.10.29		152 ±7·1		5
Untreated ,, ,,	12.10.29		133 ±9·0		0
Average		95	140		
Worcester Pearmain					
Archbold (1932)	26.8.29	243 ±10·6		0	
,, ,, ...	17.9.29	221 ±12·3		16	
Onslow, Kidd and West (1932)	5.9.27	242 ±9·3		14	
,, ,, ,, ...	8.9.27	148 ±28·6		0	
,, ,, ,, ...	9.9.27	201 ±11·2		0	
,, ,, ,, ...	12.9.27	230 ±25·9		20	
Average		214			

DISCUSSION.

The data presented for Bramley's Seedling and for Worcester Pearmain apples which were gathered at various stages of maturity during the period of their growth by cell enlargement and which were subsequently stored in air, conform to a single pattern. After an initial period during which acid does not fall and may possibly even rise, a steady logarithmic decline in acid content sets in, which is not affected by the climacteric rise in respiratory activity. In nitrogen the pattern is the same except that there is no delay period. It is therefore tentatively concluded that the process involved in the loss of malic acid is one of which the rate (per unit fresh weight) is determined by the concentration of acid in the cell sap. The rate of acid loss is increased when the tissues show visible signs of low-temperature breakdown, as previously reported by Haynes (1925).

The stage of growth (enlargement of cells) makes but little difference to the rate constant of acid loss per unit fresh weight after gathering (Fig. 2). On the other hand, there is a marked difference between the rate constant of acid loss in Bramley's Seedling apples and that in Worcester Pearmain apples, the rate constant in the latter being about twice that in the former variety. The Bramley's Seedling is a more acid fruit than the Worcester Pearmain. There is thus a relationship between the rate constant of acid loss of a variety (as determined during storage after gathering) and its characteristic acidity on the tree—a lower rate of acid loss being associated with a higher content of acid.

Bennet-Clark and Bexon (1943) produced evidence that in the beetroot malic acid is converted into a three-carbon molecule with evolution of CO_2 by decarboxylation at or near the outer surface of the cytoplasm. If this is so in apples, then amongst the factors influencing the rate of acid loss per unit fresh weight will be (*a*) the area per unit fresh weight of the inner boundary of cytoplasm and (b) the thickness of the cytoplasmic layer. Data are available (Hulme, 1949, Smith, 1950, and unpublished data) from which we have been able to calculate values for these factors for a particular population of Bramley's Seedling apples gathered in the autumn of 1936. These values are given in Table V.

TABLE V.

The area per unit fresh weight of the inner boundary of the cytoplasm and the thickness of the cytoplasmic layer of Bramley's Seedling apples (1936).

Date of gathering.	Area of vacuole/cytoplasm boundary per gm. fresh weight ($mm^2 \times 10^{-4}$).*	Thickness of cytoplasmic layer (μ).*
11.6.36	9·1	0·60
17.6.36	7·6	0·78
23.6.36	6·0	0·40
6.7.36	4·6	0·35
16.7.36	4·2	0·38
6.8.36	3·4	0·29
23.8.36	3·3	0·27
31.8.36	3·2	0·24
8.9.36	3·2	0·24
21.9.36	3·0	0·26
5.10.36	2·8	0·27
13.10.36	2·8	0·27
27.10.36	2·9	0·33

* Values for protein nitrogen for this population were obtained by Dr. A. C. Hulme, and have already been published (Hulme, 1950). Values for mean volume of cell and for mean number of cells per apple were obtained for the same population of apples by Mr. W. H. Smith (personal communication). Protein nitrogen is converted into volume of hydrated protoplasm by applying the factors 5 and 6·8. The volume of the hydrated protoplasm is subtracted from the volume of the cell to give the volume of the vacuole. Treating these two volumes as spheres, the radii are calculated, and the difference between the radii is taken as the mean thickness of the cytoplasmic layer of the cell. The area of the vacuole/cytoplasm boundary is the calculated surface of the vacuolar sphere.

It can be seen that from mid-June to full maturity in early October the thickness of the cytoplasmic layer and the area per unit fresh weight of the vacuole/cytoplasm interface diminished by about the same extent (3 : 1). Decreased thickness of the cytoplasmic layer will, by shortening the diffusion path, accelerate the arrival of malic acid at the outer surface of the cytoplasm. Decrease of the area of the vacuole/cytoplasm interface (per unit fresh weight) will decrease the amount of malic acid (per unit fresh weight) reaching the surface of the cytoplasm.

In the course of development, therefore, the effects of change in the thickness of the cytoplasmic layer and in the area of the vacuole/cytoplasm interface will tend to offset one another. This suggestion is put forward to account for the fact that the rate constant of loss of acid after gathering does not change during the course of development by cell enlargement of the apple on the tree.*

Data obtained by Smith (in the press) for cell size and number in Worcester Pearmain apples are also available, and from these the two varieties can be compared at maturity (Table VI). We are indebted to Dr. Hulme (personal communication) for protein-nitrogen values for four populations of Worcester Pearmain apples. These values were respectively 0·023, 0·023, 0·022 and 0·022 grammes per 100 grammes fresh weight. These populations represent two seasons and three localities.

TABLE VI.

The area per unit fresh weight of the inner boundary of the cytoplasm and the thickness of the cytoplasmic layer of Bramley's Seedling and Worcester Pearmain apples at maturity.

Variety.	Area of vacuole/cytoplasm boundary per gm. fresh weight (mm$^2 \times 10^{-4}$) at maturity.	Thickness of cytoplasmic layer at maturity (μ).
Bramley's Seedling	2·8	0·27
Worcester Pearmain ..	3·8	0·18

In this comparison it is seen that both the thickness of the cytoplasm and the area of the vacuole/cytoplasm interface are such as might account for the rate constant of acid loss (per unit fresh weight) being greater in the Worcester Pearmain than in the Bramley's Seedling apples.

We have next to consider the delay period and its significance. The facts appear to be (1) that the delay period does not occur when oxygen is absent or present in very low concentration, (2) that it is less marked in mature fruit than in less mature fruit, and (3) that it is less marked in Worcester Pearmain than in Bramley's Seedling apples. The first two facts suggest that the delay period is due to a formation of acid involving oxidation and continuing for a while after gathering until an essential precursor is exhausted. If this explanation is valid it implies that after gathering and for the duration of the delay period acid is being formed at a rate approximately the same as that at which it is destroyed and that the two processes are independent.

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* In our experiments the rate constant for acid loss is determined in gathered fruit after the disappearance of starch. When starch is present in the fruit on the tree, it may, at its maximum, amount to 1 to 2 per cent. of the fresh weight of the fruit. The volume of the starch present in the cytoplasm will then be about the same as the volume of the cytoplasm.

TABLE VII.

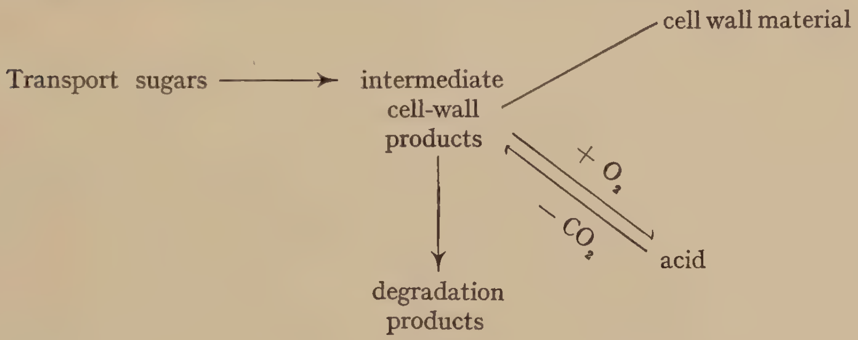
Instantaneous rate of accumulation in the apple of acid, cell-wall and nitrogen proceeding on the tree prior to gathering, and a calculated rate of loss of acid.

(*Expressed as gm. per day per* 100 *gm. of fresh weight on mean weight of apple during the period.*)

Experiment.	*Malic acid.*				*Cell-wall.* (Alcohol-insoluble residue other than starch).		*Nitrogen.* (as protein).	
	Rate of increase.		Rate of loss.		Rate of increase.		Rate of increase.	
Gathering dates.	Bramley's.	Worcester.	Bramley's.	Worcester.	Bramley's.	Worcester.	Bramley's.	Worcester.
H. K. Archbold (1932).								
11.6 → 17.6	0·250	0·165	0·010	0·011	0·550	0·726	0·153	0·135
17.6 → 24.6	0·241	0·076	0·012	0·012	0·314	0·191	0·062	0·021
24.6 → 3.7	0·141	0·080	0·014	0·012	0·204	0·264	0·032	0·029
3.7 → 14.7	0·058	0·055	0·014	0·012	0·070	0·200	0·011	0·021
14.7 → 28.7	0·037	0·017	0·012	0·009	0·050	0·104	0·007	—
28.7 → 11.8	0·032	0·008	0·011	0·007	0·047	0·068	0·005	0·001
11.8 → 26.8	0·001	0·008	0·010	0·006	—	0·036	0·002	—
26.8 → 8.9	0·004	—	0·009	—	0·028	—	0·001	—
8.9 → 22.9	0·007	—	0·008	—	0·012	—	0·002	—
22.9 → 7.10	0·010	—	0·008	—	0·009	—	0·002	—
7.10 → 21.10	0·006	—	0·008	—	0·005	—	0·002	—
A. C. Hulme (1950)								
11.6 → 23.6	0·199						0·051	
23.6 → 6.7	0·105						0·019	
6.7 → 16.7	0·041						0·011	
16.7 → 6.8	0·019						0·002	
6.8 → 23.8	0·009						0·003	
23.8 → 31.8	0·014						0·002	
31.8 → 8.9	—						0·001	
8.9 → 21.9	0·011						0·001	
21.9 → 5.10	0·011						0·002	
5.10 → 13.10	—						—	
13.10 → 27.10	—						0·001	

Table VII shows the instantaneous rate of acid accumulation (gm./100 gm. F.W./day), based on data of Archbold (1932) and of Hulme (1950), which was proceeding during growth on the tree prior to gathering, and a calculated rate of loss assumed, on the basis of our data, to be also proceeding. The rate of increase of acid on the tree is much larger than the calculated rate of loss. The uptake of water and the increase in cell size more than balance the formation of acid so that the acid concentration in the cell sap does not change quickly or to any great extent. It is about 1 per cent. at the beginning of June; reaches a peak value of 2 per cent. early in July; and falls to about 1 per cent. towards the end of October. With the cessation of entry of water and solutes from the tree by translocation, which must follow the picking of the apple, the rate of increase of acid must fall abruptly, otherwise there would be a marked rise in acid concentration after gathering.

The delay period and the sudden fall in the rate of accumulation of acid after gathering might be expected to occur if, in the growing apple on the tree, acid was being formed in a system of the following character:



In such a system increase of acid takes place mainly as the result of an increase in the volume of the vacuole by addition of water. At gathering, transport ceases but cell wall intermediates continue to give rise to acid at a more or less constant rate to balance acid loss by decarboxylation until these intermediates are exhausted through degradation. Our data (Griffiths and Potter, 1950) for the changes in the alcohol-insoluble cell wall material after gathering indicate that there is in fact at the time of gathering a fraction of this material which is degraded into a soluble form during a short initial period.

Archbold (1932), studying the growth of Bramley's Seedling and Worcester Pearmain apples, came to the conclusion that acid formation on the tree was linked either to protein formation or to cell-wall formation, because of the parallelism exhibited between the progressive changes during development in the rates of increase of these three components. They all decline after reaching a peak value early in the growth period. It appears, however, from Table VII that the ratio of the rate of cell-wall formation to the rate of acid accumulation is different in the two varieties, Bramley's Seedling and Worcester Pearmain. On the other hand, the ratio between the rate of protein increase and the rate of acid increase is nearly the same. It might, therefore, be suggested that the delay period is due to a continuous formation of acid linked with a continuous formation of protein. Hulme's (1937) data show, however, that up to the middle of August, net degradation of protein takes place in an adjustment phase in apples after gathering, while after the middle of August the adjustment phase is one of net formation of protein. On the whole, therefore, we are inclined to regard the formation of malic acid and its secretion into the vacuole as being involved, as a side reaction, in the process of cell-wall metabolism, and we think the delay period must be accounted for on the lines suggested above. Further, we look upon this system as being analogous to that in which starch is concerned, in so far as both cell wall and starch appear to be net deposits of surplus carbohydrate—deposits which draw on the transport sugars for their income, but which are continuously undergoing degradation.

That the delay period is less marked in the case of Worcester Pearmain than in the case of Bramley's Seedling apples is, on our present view, to be associated with the fact that the cells of the Worcester Pearmain apple are more active in the formation of cell wall than are the cells of the Bramley's Seedling apple, and also with the fact that the cells of the Worcester Pearmain variety are specifically more active in removing acid by decarboxylation.

Concentration of titratable acid (as per cent. of original fresh weight) during growth on the tree.

Two sets of data for the titratable acid during growth on the tree for single populations of Bramley's Seedling apples are available, that of Archbold for the 1930 season and that of Hulme for the 1936 season. Immediately after petal fall (20th May approximately) the concentration of titratable acid is low. It reaches its peak values about the 27th to 30th June and then falls steadily. The peak values are 2 per cent. for the 1930 population, and 1·6 per cent. for the population of 1936, and this order of difference is maintained throughout. We have single points for the main experiments dealt with in this paper, and if we assume that they fall on regular curves of the type shown by the data of Archbold (1932) and Hulme (1950) we can construct the series shown in Fig. 5. From this figure we conclude that in different populations, in different localities and in different seasons there is a wide scatter as regards the characteristic titratable acidity of the developing fruit, and that it may be possible from observations of the titratable acidity at any stage to forecast what it would be at maturity.

While studies by the titration method of changes in acid present after gathering are not likely to be upset by changes in the amount of alkali present (Kidd and Hanes, 1937), comparisons of the titratable acidity of different populations, from different orchards and in different seasons cannot be directly interpreted as differences in the malic acid content because the amount of base present may also vary. A further study of this question by methods in which the cations present can be determined, is required.

Effect of temperature.

The effect of temperature upon the rate constants of acid loss is shown in Fig. 3. The effect of temperature upon the rate of CO_2-production is shown in Table I and Fig. 6.

It is seen that different populations of Bramley's Seedling apples in different seasons exhibit differences in the relation between temperature and rate of CO_2-production. This is a point which has not before been recorded. The temperature

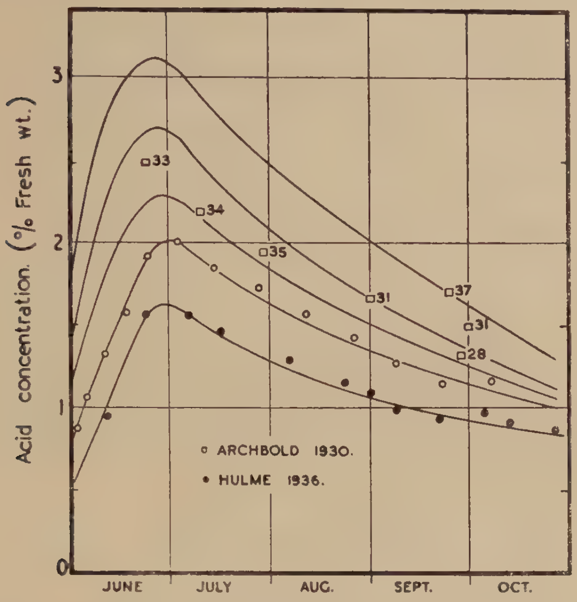


FIG. 5.

Changes in the amount of titratable acid (as malic) present in single populations of Bramley's Seedling apples during growth on the tree.

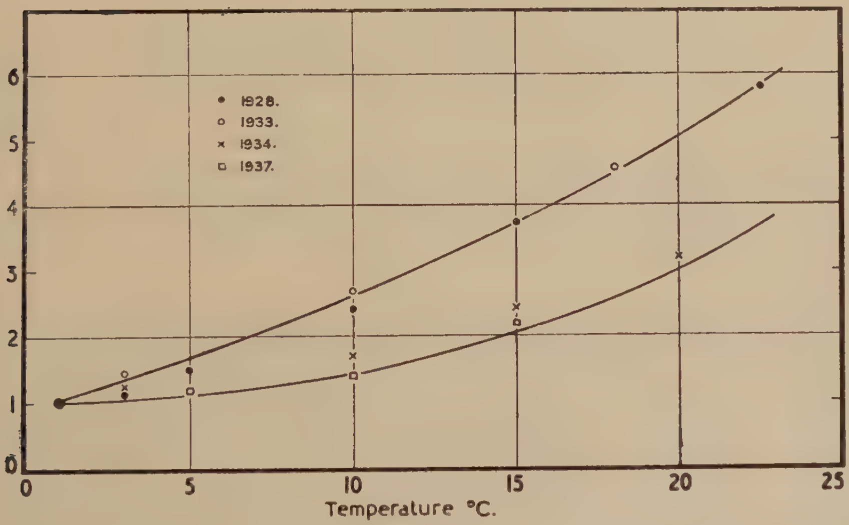


FIG. 6.

Relative rates of initial respiration of Bramley's Seedling apples taking rate at 1° C. as unity.

coefficient for the populations gathered on 10.7.34 and 24.9.37 is not much more than one-half that of the other populations.

The relationship between rate of acid loss and temperature does not show this variation with population. It contrasts, however, with the relationship between rate of CO_2-production and temperature in that the increase in rate with rising temperature is smaller below 15° C. and larger above 15° C.

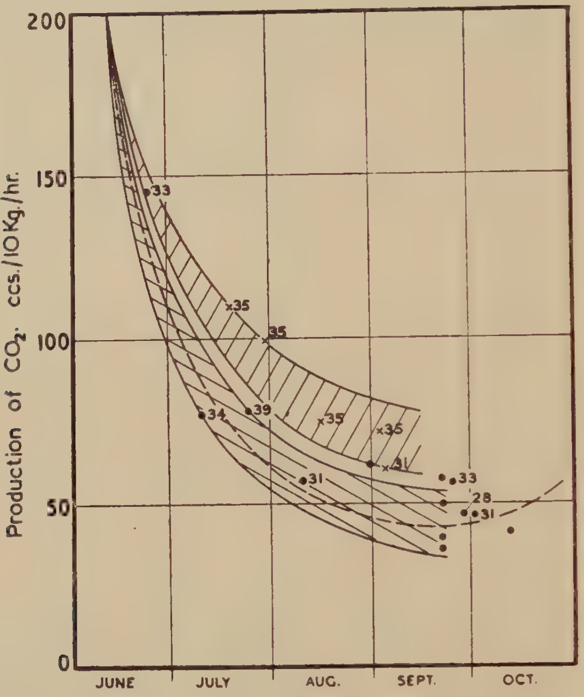


FIG. 7.

" Initial " respiration rates after gathering.

- Various populations of Bramley's Seedling apples (see Tables I and IV) at 10° C.

× Various populations of Worcester Pearmain apples (see Table I) at 10° C.

— — — Single population of Bramley's Seedling apples (1936) at 12° C. Hulme's data.

CO_2-production.

Hulme (in the press) has shown that for any one population of Bramley's Seedling apples the rate of CO_2-production (per 100 gm. fresh weight), which falls during development on the tree, bears a constant relation to the amount of protein nitrogen present per 100 gm. fresh weight, from which the deduction is made that CO_2-production is approximately proportional to the amount of cytoplasm present. From our data a comparison can be made between the rate of CO_2-production (per 100 gm. fresh weight) of Worcester Pearmain apples and that of Bramley's Seedling apples throughout the main period of development. It is seen (Fig. 7) that the rate of CO_2-production per unit fresh weight of Bramley's Seedling apples compared with that of Worcester Pearmain apples is as about 1 to 1·4. This difference is not due to a difference in the *amount* of cytoplasm present per unit fresh weight, which is, in fact, not very different in the two cases. Hulme (in the press) finds the rate of CO_2-production per unit protein to be about 3·5 and 4·5 mg. CO_2/apple/100 hr. for Bramley's Seedling and Worcester Pearmain apples respectively.

The available data (Archbold, 1932 and Smith, 1950) indicate that the two varieties increase in size at about the same rate. Smith's results show that Worcester Pearmain apples gathered in September are slightly smaller than Bramley's, but contain about twice as many cells of approximately half the size. This indicates, in general, that there is more cytoplasmic surface per 100 g. fresh weight in Worcesters than in Bramley's throughout the growth period and, since the protein content of these varieties does not differ greatly, that there is a thinner cytoplasmic layer round the vacuole. We incline, therefore, to the view that not only the rate of acid loss but also the rate of CO_2-production are affected by a cytoplasmic diffusion or "transport" factor which may be of considerable importance in relation to the specific difference between Worcester Pearmain and Bramley's Seedling apples in rates of CO_2-production per unit fresh weight or per unit protein nitrogen.

TABLE VIII.

Constants "a" × 10^5 *of the equation* $\log c = b - at$, *with their standard errors.*

Experiment.	Change of temperature.	Days at original temperature before change.	After change of temperature during storage.	For storage at		
				10° C.	3° C.	1° C.
Bramley's Seedling						
IV. 23.6.33	From 10° to 3° C.	47	115 ± 9·7	225 ±3·8	181 ±6·5	—
IV. 23.6.33	From 3° to 10° C.	47	231 ±12·4	225 ±3·8	181 ±6·5	—
II. 31.8.31	From 10° to 1° C.	21	86 ±28·4	178 ±7·7	—	—
II. 1.10.31	From 10° to 1° C.	31	30 ±48·5	196 ±5·9	—	—
Worcester Pearmain						
III. 3.9.31	From 10° to 1° C.	8	235 ± 9·8	320 ±11·4	—	237 ±46·9
III. 3.9.31	From 10° to 1° C.	89	60 ±22·4	320 ±11·4	—	237 ±46·9
III. 3.9.31	From 1° to 10° C.	104	333 ±32·5	320 ±11·4	—	237 ±46·9

Effect of change of temperature.

In certain experiments the temperature was changed during the storage period. Table VIII sets out the results obtained as regards the effect of temperature on the rate constant. From this table it will be seen that the change of temperature studied was from 10° to 3° C. or the reverse and from 10° to 1° C. or the reverse. In all cases where a change of temperature was an upward one, the *a* value at the new temperature was practically the same as that of apples stored from the outset at the higher temperature. Where the change was a downward one, except in the case of the change made very soon after the beginning of the storage period, i.e. Worcester Pearmain apples from 10° C. to 1° C. after eight days storage, the rate constant at the lower temperature was considerably lower than the rate constant of similar fruit held at that temperature from the outset. No explanation of this phenomenon can at present be offered.

Rate of starch degradation.

It is of interest in connection with the present paper to mention briefly for the sake of comparison the results of our previous analysis of the rate of degradation of starch in apples after gathering (Kidd, West, Griffiths and Potter, 1950). The rate constant of starch degradation is higher for Bramley's Seedlings than it is for Worcester Pearmains and is little affected by the stage of development of the fruit. The temperature coefficient for the rate of starch degradation is markedly different from that for acid loss.

SUMMARY.

(1) Data for a number of populations of Bramley's Seedling apples and of Worcester Pearmain apples have been examined in respect to the changes occurring in the amount of acid present during growth and during storage after gathering at different stages of growth.

(2) The data fall into a single pattern which enables the following generalizations to be made:

(*a*) After gathering there is a delay period of varying length during which acid remains unchanged in amount within the limits of error of the observations. Subsequently it decreases in amount at a rate proportional to the concentration of acid present.

(*b*) The rate constant of acid loss does not change appreciably with the stage of growth, and appears to be a physiological characteristic of the variety.

(*c*) The rate constant of acid loss of Worcester Pearmain apples is about twice that of Bramley's Seedling apples.

(*d*) The value of the rate constant of acid loss can be related to the thickness of the cytoplasm and to the area of the cytoplasm/vacuole interface, and can thus be related to the characteristics of the two varieties as regards cell size and cell number.

(3) The loss of acid, which takes place in gathered fruit, is considered to be due to decarboxylation which does not require the presence of free oxygen. The formation of acid on the tree is considered to be due to a process requiring the presence of oxygen, and to be linked with the systems involved in cell wall formation.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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(*Received* 2/8/50.)

STUDIES OF COMPATIBLE AND INCOMPATIBLE GRAFT COMBINATIONS WITH SPECIAL REFERENCE TO HARDY FRUIT TREES

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SYNOPSIS

At the beginning of the present work the only practicable method of estimating compatibility between rootstock and scion involved the collection of data on the behaviour of the tree over a period of years, as no method of forecasting the compatibility of a graft combination was known.

Since incompatibility is sometimes overcome in practice by the use of an intermediate stem-piece of a variety compatible with both scion and rootstock, trials of double-grafted pear and plum trees with intermediate stem-pieces were designed. The object of these trials was to discover whether the time and amount of vegetative growth, the internal structure of the plant stems, the structure of the graft unions or the distribution of starch, were related to the compatibility of the graft combination. Further studies were made on a trial of single-grafted peach trees on two rootstocks, one of which was compatible and one incompatible with the scion variety.

Neither the type of vegetative growth nor the beginning of cambial activity in spring appeared to be directly associated with the causes of incompatibility. No connection appeared to exist between compatibility and the features of the stem structure that were examined. The structure of the graft unions, however, the condition of the inner bark above and below the union and the distribution of starch in the different parts of the trees were found to be related to compatibility.

INTRODUCTION.

By the practice of grafting, two living systems are brought into union, and this union provides the biologist with many interesting problems. The failure of certain combinations of plants to intergraft and the different degrees of compatibility when union takes place have received much attention. In the animal body the tissues of different species cannot be grafted together, and within the individual of a species there is a great variation in the compatibility according to the kind of tissue to be grafted. Cornea can be grafted in the human species from one individual to another, while skin grafts are only possible from one region to another on the same individual. In the plant kingdom the range of compatibility is much wider and grafting between individuals belonging to different species and even different genera is a common practice in horticulture. Nevertheless incompatibility between rootstock and scion is a well-known fact and the botanical relationship of the components of the graft is not a reliable guide to the behaviour of the graft combination. The problem has assumed a place of importance in the fruit tree industry since the demand for new rootstocks and varieties has increased and many of these new kinds present fresh problems of incompatibility.

As the whole subject of stock-scion incompatibility in fruit trees was reviewed by Argles up to 1937, the present introduction will deal mainly with the work done since that date. The diversity of factors mentioned in the literature as responsible for, or related to, incompatibility indicates the complexity of the problem. The following factors were considered to be among the more important by various workers between about 1890 and the time of Argles's review in 1937 : differences in seasonal periods of growth and in vegetative vigour, biochemical differences, mechanical blockage at the union, different rates of callusing and precipitin reactions.

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The evidence that a virus may be responsible for the failure of certain stock-scion combinations is more recent. Toxopeus (1936) reported an interesting case of incompatibility in citrus between sweet orange (*Citrus sinensis*) and sour orange (*Citrus aurantium*) and suggested as a result of his experiments that the scion produced some substance toxic to the stock. The combination was incompatible in Java and South Africa although it was compatible in a number of other localities. During the last few years great attention has been paid to quick spreading diseases involving the sour orange rootstock. The diseases were called " tristeza " in South America (Bertelli, 1944, 1947), " quick decline " in California (Fawcett, 1946) and " bud union decline " in Australia (McAlpin *et al.*, 1948). The cause of these diseases has been pointed out to be of a virus nature (Fawcett and Wallace, 1946, Meneghini, 1946) and the parallelism between them has often been established, suggesting that a similar infectious agent is responsible (Olmo and Moreira, 1947, McAlpin, 1948).

Oberholzer (1947) indicated as a result of his experiments that the incompatibility reactions observed in South Africa, particularly so far as the sour orange is concerned, are probably directly related to the problem of " tristeza " and " quick decline " and so probably due to virus. This virus would seem to be present in a latent form in certain species or varieties, and to set up pathological conditions only when certain stock-scion combinations are made. The virus would seem to be infectious, being distributed in other ways as well as by grafting. In the cases of " tristeza " the existence of an insect vector is known (Meneghini, 1946, 1948).

A similar case to the one described in Citrus has been reported in apples. Shaw and Southwick (1944) reported that the clonal stock Spy 227 proved to be incompatible with certain varieties of apples and with certain strains of the variety McIntosh which otherwise appeared to be identical. Tukey and Brase (1944) reported a similar case with two other strains of McIntosh, and Yerkes and Aldrich (1946) with strains of Winesap. Gardner and others (1946) pointed out that the phenomenon bears a striking parallelism to that of " tristeza " disease of Citrus, and that a virus could be responsible for it. Finally Weeks (1948) has demonstrated that a toxic principle causing the death of Spy 227 can be transmitted by grafting from a lethal strain to a congenial strain, the principle not being manufactured in the leaves. Weeks concludes, " It would appear from the evidence at hand, that the toxic principle is a virus. If it is a virus it is of a most peculiar nature, in that it only manifests itself when two comparable factors are brought together. There is no evidence that this virus has a vector."

A similar case has been reported in relation to a known virus. When sweet cherries infected with " buckskin disease " are grafted on Mahaleb stock, the stock becomes infected and dies (Thomas and Rawlins, 1941). On the other hand when the sweet cherry is on Mazzard roots the tree is not seriously affected by the virus disease (Rawlins and Parker, 1934). Schneider (1945) devoted more attention to this case.

Apparently very little work has been carried out in order to discover whether there is any relationship between the histological structure of the components of a grafted tree and their compatibility, although the suggestion that uncongenial unions may be due to differences in the size of the cambial cells has been made by Bradford and Sitton (1929). Correlations between the histological structure of apple rootstocks and the growth and fruiting of scion varieties grafted on them have been described by Beakbane and Thompson (1939 and 1947). The methods of examination used for the apple rootstock work have been adopted in the present studies on histological structure as related to compatibility.

For the present work three field trials were designed to study compatible and incompatible combinations during their first two years' growth. Trial I, " plum compatibility " and Trial II, " pear compatibility " were composed of double-grafted trees, both compatible and incompatible varieties being used in various combinations on one rootstock. Trial III, " peach compatibility " consisted of single-grafted trees of one variety on two rootstocks showing different degrees of compatibility.

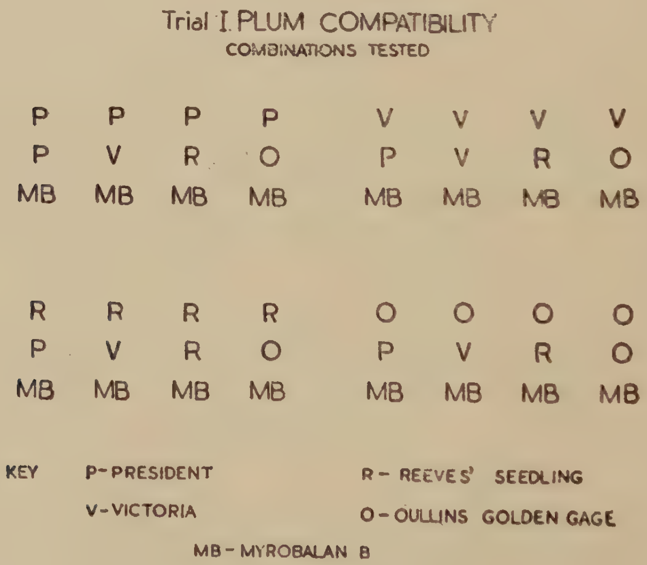
PLANNING AND PLANTING.

Trial I. Plum compatibility.

In the plum trial the rootstock chosen was Myrobalan B (a clone of *Prunus cerasifera**) ; the varieties included were two which are normally compatible with this rootstock when single-grafted, viz. Victoria (Amos *et al.*, 1936) and President (Hatton, 1936), and two which are usually incompatible, viz. Oullins Golden Gage (Hilton, 1939) and Reeves' Seedling (unpublished work at East Malling Research Station), Fig. 1.

A hundred and ninety-six rootstocks were planted in the winter of 1946-47 at a distance of twelve inches apart in a single row.

The arrangement of the scions was as indicated in Fig. 2. Four replicates of each scion-intermediate combination were included using three-tree plots.



Trial I. PLUM COMPATIBILITY

COMBINATIONS TESTED

P	P	P	P	V	V	V	V
P	V	R	O	P	V	R	O
MB	MB	MB	MB	MB	MB	MB	MB
R	R	R	R	O	O	O	O
P	V	R	O	P	V	R	O
MB	MB	MB	MB	MB	MB	MB	MB

KEY P-PRESIDENT R- REEVES' SEEDLING

V-VICTORIA O-OULLINS GOLDEN GAGE

MB- MYROBALAN B

FIG. 2.

Diagram showing arrangement of varieties in double-grafted trees.

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* The nomenclature is according to Rehder (1947).

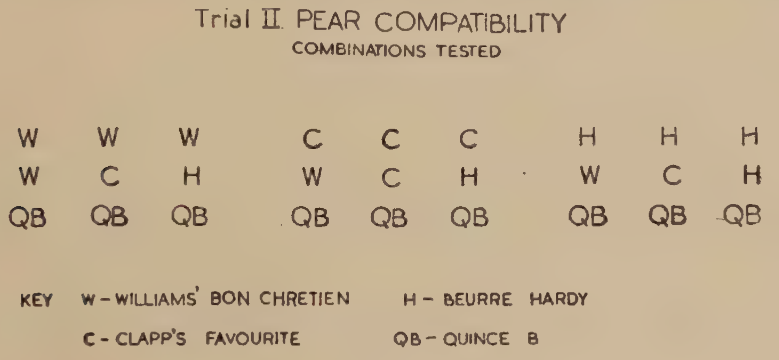
In March, 1948, the double grafting was carried out in one operation by the method described by Garner (1944). As soon as both unions had "taken", the leaves and buds on the intermediate were removed so that each tree was formed of three parts: (1) the top scion, providing the leaves and most of the stem of the tree, (2) the intermediate, providing a piece of stem of about five inches, (3) the rootstock, providing the base of the stem for a length of about three inches, in addition to the root system.

The pruning and cultivation treatments were those normally used at the Station for plum tree nurseries.

Trial II. Pear compatibility.

The rootstock chosen for this trial was Quince B (a clone of *Cydonia oblonga* Mill.) and the scion varieties included were Beurré Hardy, Williams' Bon Chrétien, and Clapp's Favourite; the first variety being considered compatible, and the last two incompatible with quince roostocks (Garner, 1947 and 1948). Beurré Hardy is also used as an intermediate to overcome incompatibility (Garner, 1944).

The arrangement of the scions is illustrated in Fig. 3. Six replicates of each scion-intermediate combination were included using three-tree plots. The trees were grafted on a total of one hundred and sixty-four rootstocks planted in a single row.



W	W	W	C	C	C	H	H	H
W	C	H	W	C	H	W	C	H
QB	QB	QB	QB	QB	QB	QB	QB	QB

KEY W - WILLIAMS' BON CHRETIEN H - BEURRE HARDY
C - CLAPP'S FAVOURITE QB - QUINCE B

FIG. 3.

Diagram showing arrangement of varieties in double-grafted trees.

The distance of planting, method of grafting, and treatments during 1948 were the same as those used in Trial I.

In March, 1949 the trees were headed back at 2 ft. 6 in. and the stem disbudded up to the six top buds.

Trial III. Peach compatibility.

The peach variety, Hale's Early, was single grafted on two different rootstocks, viz. Myrobalan B and Brompton (a clone of *Prunus domestica*). The first combination is considered to be incompatible and the second to be compatible (Witt and Garner, 1931).

During the winter of 1946-47 the rootstocks were planted in two rows at 12 in. by 4 ft. Fifty trees of each combination were grafted in March, 1948, by the whip and tongue method.

FIELD OBSERVATIONS AND LABORATORY STUDIES.

The following description is divided into two parts : (*a*) the records and observations made in the field and, (*b*) the studies carried out in the laboratory with material from the field trials.

The exposition in parts *a* and *b* is arranged chronologically.

Abbreviations are frequently used for the graft combinations, for example Hale's /Brompton represents a tree of Hale's Early peach grafted on Brompton rootstock and V/O/MB represents a double-grafted tree composed of Victoria grafted on Oullins Golden Gage on Myrobalan B rootstock.

(*a*) *Records on growth and on cambial activity.*

Trial 1. Weekly observations on the most advanced bud were made on the top-scion and intermediate of each combination during April and May, 1948, and the stage of development of the bud assigned to one of the five classes described below.

Stage 1.	Bud dormant. No swelling.
Stage 2.	Bud swelling. The brown and green areas produced by the scales and leaves on the bud giving the impression of half green and half brown. No leaf tips visible at the top of the bud.
Stage 3.	More than half of some leaves visible.
Stage 4.	Bud fully open. No elongation of stem.
Stage 5.	New stem beginning to show.

A record of the percentage " take " for each combination was made in May, 1948.

The girth of the stock and the total height of the trees were measured monthly during the first year's growth (1948).

A record of autumn defoliation was made at the end of the first year's growth (November, 1948) by estimating in ten categories the percentage of leaves dropped.

During the spring of 1949 observations were made on the initiation and basipetal spreading of cambial activity in one tree per combination. The " strip " method described by Priestley (1933) was used. At the positions on the tree stem where the observations were made the bark was carefully peeled off in a rectangle of about 2 sq. cm. The new cells laid down by the actively growing cambium on the lignified wood of the previous season exposed by the removal of the bark were stripped with a small chisel. The rectangle of bark was replaced and tied with raffia. The strips were floated on water and their thickness and cohesion recorded. When the observations began in April, the first place tested was immediately below the apical bud. As soon as a strip could be collected in this position, lower portions on the stem were examined until a position was reached where no strip could be collected. Observations were repeated at intervals of about ten days and notes on the stage of development of the buds were made at the same time.

In 1949, in the second year's growth, the girths of the stock, intermediate and top-scion were measured at the beginning of the growing season (May) and at the end (October). The position at which the measurements were made was as follows : (i) on the rootstock in the same position as in the preceding year ; (ii) on the intermediate half way between the upper and lower union, and (iii) on the top-scion at about two inches above the union.

A monthly record of the length of the six terminal shoots of each tree was made from May to September, 1949.

Trial II. The same records as in Trial I were made using the same methods.

Trial III. The percentage " take " was recorded in May, 1948.

The girth of the stock and the length of the main shoot of the scion were recorded monthly during the first growing season (1948).

A record of the autumn defoliation was made in October, 1948.

Observations on the cambial activity of the scion and the stock were made during the spring of 1949.

The methods used were as described in Trial I.

(*b*) *Histological studies and starch distribution.*

Trial I. Two trees of each of the sixteen combinations in this trial, were used for histological examination when they had completed one year's growth since grafting (winter 1948-49). The following sections were made :

(1) transverse sections in the quadrant of the stem occupying a west position in the plot as follows : Section 1 top-scion above the union ; Section 2 intermediate half way between the unions ; Section 3 rootstock stem, below the union, and Section 4, thickest lateral root at a distance of about two inches from the junction of the root with the stem.

(2) radial longitudinal sections of the stem at the two unions, i.e. Section 5 at the union between top-scion and intermediate and Section 6 at the union between intermediate and rootstock.

The exact position of each section was marked on a scale drawing of the appropriate part of the tree (see Fig. 4). The method of sectioning is described below.

The transverse sections in combinations V/V/MB, P/P/MB, O/O/MB, R/R/MB, were used for detailed studies of the histological characteristics of the wood and of the cambial zone in order to discover whether there was a correlation between histological structure and compatibility. A description was made of the appearance of the radial longitudinal sections of the unions.

The starch content of the different parts of the tree was estimated at the time of sectioning by placing the sections in a 0·3 per cent. solution of iodine in 1 per cent. potassium iodide.

In addition the starch content of radial longitudinal sections of the upper and lower unions was recorded in March, 1949, for one tree per combination, and in October, 1949, for two trees per combination.

Finally four trees per combination were used for macroscopical examination of the unions. The unions were sawn longitudinally, the bark peeled off and a description of each union made.

Trial II. The methods of choosing and making the sections and of making the observations on starch were similar to those used for Trial I. Some of the histological characteristics were studied in two trees per combination, while others were examined only in the combinations H/H/QB, W/W/QB and C/C/QB.

Trial III. Histological characteristics in the wood and cambium of stock and scion were studied in two trees per combination during the winter 1948-49. Records of the amount of starch were made at various times during the first and second year's growth.

Method of sectioning.

Sections of from fifteen to forty microns thick according to the kind of tissue, were cut from fresh material with a sledge microtome. After they had been stained in a 1 per cent. solution of Safranine in 50 per cent. alcohol, the sections were gradually washed and dehydrated in alcohol and then placed in a mixture of alcohol and clove oil in equal proportions by volume, and stained in 2 per cent. Light Green in the same mixture. The sections were then washed in a mixture of alcohol and clove oil and finally placed in pure clove oil, cleared in xylol and mounted in Canada Balsam.

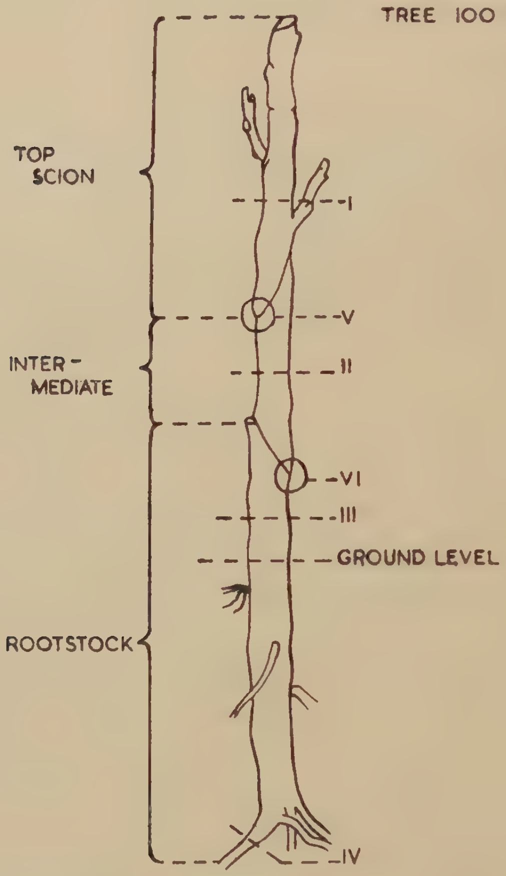


FIG. 4.

Diagram of a double-grafted tree to show the relative positions of transverse and radial longitudinal sections. The positions of the latter are indicated by circles.

Histological characteristics studied and methods used.

Wood. The method described by Beakbane (1941) was used. As the wood structure varies according to the period of the year in which it grows, the middle of the latest formed annual ring* was chosen for the samples. In each section three microscopic " fields " of 92,000 sq. μ were chosen at random.

A photomicrograph on bromide paper, having a magnification of 440 diameters was obtained of each " field " with a Vickers projection microscope. Fig. 11 shows some of the photographs from which studies were made of, (1) the relative areas occupied by the four elements (fibres, vessels, parenchyma cells and rays) composing the wood and, (2) the size of fibres, vessels and parenchyma cells. The photomicrographs were cut up into each of the four elements which were weighed separately, and the percentage area of each group was estimated from the weights so obtained.

The number of fibres, vessels and parenchyma cells was counted in each photograph and an estimate of the mean weight of the photograph of a single cell was obtained. By calculating the area corresponding to unit weight of bromide paper, the mean weight of the photograph of a fibre, a vessel and a parenchyma cell was converted into area, giving an estimate of relative cell size. The size of the ray cells was not calculated by this method, because it was not possible to count them with a sufficient degree of accuracy, but the width of the rays was recorded as described below.

Cambial zone. The width of the rays and the distance between rays were measured along the cambial perimeter, as seen in transverse section, with the aid of an eyepiece micrometer. The number of rows of cells forming each ray was also recorded. From these measurements the following information was obtained:

(1) The average width of uniseriate and multiseriate rays.
(2) The average distance between rays.
(3) The relative numbers of uniseriate and multiseriate rays.

Method of analysis.

Similar data have already been analysed statistically (Beakbane, 1941); the method employed being the analysis of variance, assuming that the variability is the same for every observation in an analysis.

The two trees per combination for the histological study were selected in a regular way from randomized blocks in the field trial, and for each tree the sections were always taken in similar places as has been described (Fig. 4).

Many of the characteristics studied are expressed as percentages. The variance of percentages is not stable throughout their range, and it is convenient, for the purpose of analysis, to transform them to some function of the percentage, such as the quantity θ where

$$\sin^2 \theta = \frac{\text{percentage}}{100}$$

which gives a similar variation throughout the range (Bliss 1937 and 1938).

As the present investigation was concerned with the question of whether incompatible combinations were associated with a different structure above and below

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*The interpretation of the terms used is that given in the *Glossary of Terms used in Describing Woods*, published by the Committee of Nomenclature of the International Association of Wood Anatomists.

the union, the characteristics studied for each combination were tested for significance in the following way :

Trial I. (*a*) Section 1 (top-scion) versus Section 2 (intermediate).

(*b*) The means of Sections 1 and 2 versus Section 3 (stem of the rootstock).

The reasons for taking the mean of Sections 1 and 2 in the second comparison was that both belonged to the same variety.

Trial II. Some of the histological characteristics were studied only in the combinations having the same variety as intermediate and top-scion, while others were studied in every combination present in the field trial. For the former the test was as in Trial 1. For the latter it was (*a*) Section 1 (top-scion) versus Section 2 (intermediate), (*b*) Section 2 (intermediate) versus Section 3 (stem of the rootstock).

Trial III. As only single grafted trees were included here, the test was scion versus rootstock.

A " t " test using the probability level $P = 0{\cdot}05$, was carried out in each case. The analysis was carried out as for a randomized block experiment, each tree being a replicate.

The possible influence of the plum and pear varieties included in Trials I and II, on the structure of the rootstocks Myrobalan B and Quince B was also studied. Since the trees of each combination had been selected from a pair of blocks of the field trial, the results were analysed in the customary way for randomized block experiments. " F " tests were carried out to test for possible effects of the scion on Section 3 and on Section 4.

RESULTS.

PLUM AND PEAR COMPATIBILITY TRIALS WITH DOUBLE-GRAFTED TREES. (TRIALS I AND II.)

Growth and other field observations.

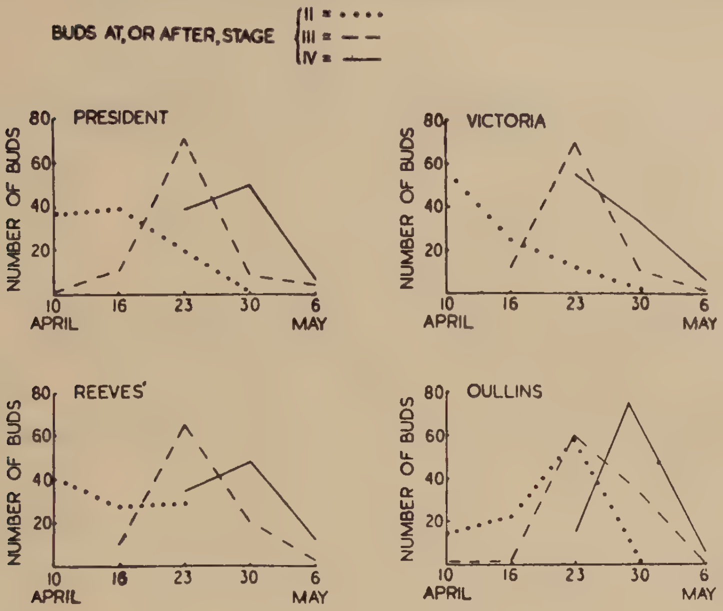
(1) Bud-break.

The data on the time of opening of the vegetative buds of the top scions and intermediate stem-pieces were examined in relation to varietal differences. The graft wood of all varieties was collected at the same time, stored in the same place and used at one period only for grafting all the combinations. If, therefore, differences in bud-break occurred between the groups formed by the varieties, they could be assumed to be due to varietal characteristics rather than to variation in treatment.

The diagrams in Fig. 5 show the number of buds reaching or passing the stages, 2, 3 and 4 at certain periods during the spring. The bud-break stages are defined above. It should be noted that the number of buds observed was the same for each variety. Varietal differences in bud-break were small in the spring following grafting and those occurring did not appear to be correlated with incompatibility since the curves for the varieties Reeves' and President and those for the varieties Clapp's and Hardy are similar within each pair of varieties, while their degree of compatibility with the rootstocks used is different.

Trial I. PLUM COMPATIBILITY

BUD DEVELOPMENT ON SCION AND INTERMEDIATE FOR EACH VARIETY (1948)



Trial II. PEAR COMPATIBILITY

BUD DEVELOPMENT ON SCION AND INTERMEDIATE FOR EACH VARIETY (1948)

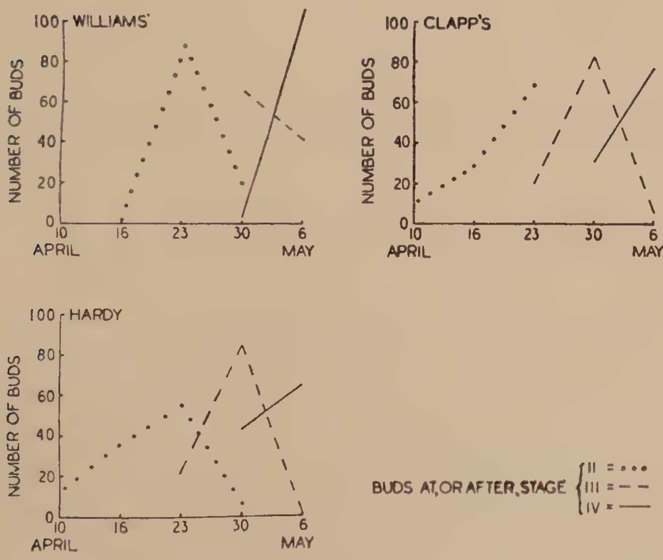


FIG. 5.

Diagrams showing number of buds reaching or passing different stages in bud-break at the date of the observation for each variety. Stage II, bud swelling, stage III more than half of some leaves visible, stage IV bud fully open.

(2) " TAKE " OF GRAFTS.

The percentage " take " of grafts is shown in Tables I and II. Although the " take " was high in every combination, the few cases of graft failure occurred in combinations containing at least one variety that was incompatible with the rootstock. This result is in accordance with the work of Chang (1938).

TABLE I.

Plum compatibility.

" Take " of grafts, height of tree and autumn defoliation, 1948, and shoot growth, 1949.

Combination.	1948.			1949.
	" Take " of grafts %	Height cm.*	Autumn defoliation.*‡	Shoot growth cm.†
P/P/MB	100	215	3	544 (6)
P/V/MB	100	215	4	560 (7)
P/R/MB	92	221	4	495 (4)
P/O/MB	100	202	4	497 (5)
V/P/MB	100	271	8	626 (9)
V/V/MB	100	250	8	622 (8)
V/R/MB	100	227	8	499 (4)
V/O/MB	100	265	8	615 (7)
R/P/MB	100	187	8	420 (8)
R/V/MB	83	175	8	407 (6)
R/R/MB	75	203	7	510 (4)
R/O/MB	100	138	8	433 (7)
O/P/MB	100	196	7	463 (6)
O/V/MB	92	188	7	437 (5)
O/R/MB	83	193	8	461 (4)
O/O/MB	92	170	8	354 (7)

* The figures in these columns are the means of twelve trees.
† The figures in brackets are the numbers of trees of which means were taken.
‡ The defoliation was recorded in categories 1 to 10. 1 = few leaves fallen ; 10 = all leaves fallen.

(3) VEGETATIVE GROWTH.

(a) *Shoot growth.*

Height of tree at the end of the first year's growth (1948) and shoot growth during the second growing season are given in Table I and II.

(i) *Varietal differences.* For the examination of varietal differences only combinations with top scion and intermediate of the same variety were used. There appeared to be no correlation between the relative amount of growth and compatibility since Victoria and President made more extension growth than the incompatible varieties Reeves' and Oullins both in the first and second years (Table I), while Williams', which is incompatible with the rootstock used, made more growth than the compatible Hardy in the second year (Table II).

Varietal differences in period of growth are given in Figs. 6 and 7. The growth periods appeared to bear no relation to the compatibility of the combination, as the most compatible varieties in Trial I, Victoria and President, have somewhat different

TABLE II.

Pear compatibility.

" Take " of grafts, height of tree, and autumn defoliation, 1948, and shoot growth, 1949.

Combination.	1948.			1949.
	" Take " of grafts %.	Height cm.*	Autumn defoliation.*‡	Shoot growth cm.†
W/W/QB	100	132	10	399 (13)
W/C/QB	100	137	10	394 (15)
W/H/QB	100	143	10	410 (13)
C/W/QB	100	111	10	354 (15)
C/C/QB	100	108	10	343 (14)
C/H/QB	94	108	10	352 (12)
H/W/QB	100	150	5	373 (14)
H/C/QB	100	131	6	306 (14)
H/H/QB	100	151	5	390 (14)

* The figures in these columns are the means of twelve trees.

† The figures in brackets are the numbers of trees of which means were taken.

‡ The defoliation was recorded in categories 1 to 10. 1 = few leaves fallen; 10 = all leaves fallen.

periods of growth while Hardy and Williams', one of which is compatible and one incompatible, had very similar growth curves (Fig. 6).

The sudden cessation of growth during August in 1949 (Fig. 7) was undoubtedly due to the exceptionally dry conditions. The rainfall during July and August was 0·54 in. and 1·36 in. respectively compared with an average rainfall of 2·20 in. and 2·27 in. for the thirty years from 1915 to 1945.

(ii) *The influence of the intermediate on the growth of the top scion.* The influence of the stem-piece of the intermediate variety on the behaviour of the top-scion in compatible combinations has been described by several authors (Grubb, 1939; Knight, 1927; Vyvyan, 1938).

In the present experiments, in addition to the normal effect of the intermediate, its " stock effect ", on the growth of the top-scion, another source of influence may have been introduced where the use of an intermediate caused a change in the compatibility of the combination. For example, the reduction in vigour of the combination V/R/MB as compared with V/V/MB (Table I) may be due to a normal dwarfing effect of the intermediate Reeves', or to the incompatibility introduced by Reeves' or to both of these causes.

Diagrams to illustrate the effect of the intermediate on the monthly growth increment were drawn in the following way:—in each group of trees with the same top-scion, the combinations in which the intermediate differed from the top-scion were compared with those where the top-scion and intermediate were of the same variety and the variation represented graphically (Figs. 8, 9 and 10). The time at which the intermediate exercised its greatest influence on growth depended on the top-scion. The maximum and minimum periods of growth occurred, in general, at the same time for all combinations with the same top-scion, although the amount of growth at each period varied with the intermediate.

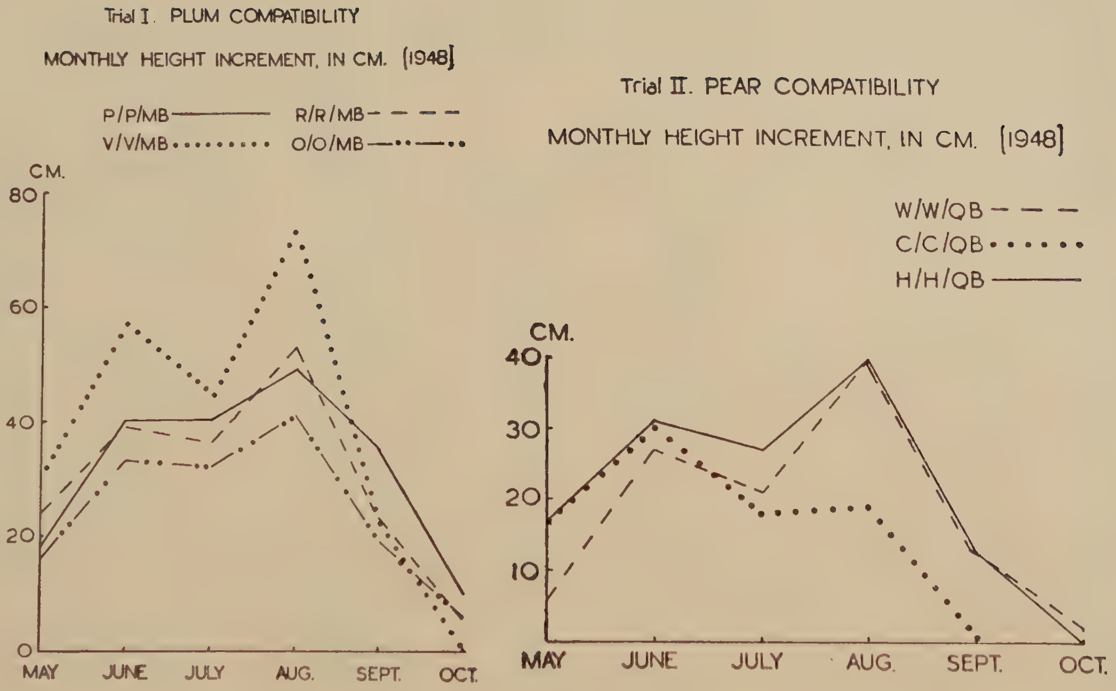


Fig. 6.

Monthly increment in growth of terminal shoots of plum and of pear/quince combinations.

It is interesting to note that the growth of the Reeves' trees is depressed by all three intermediates compared with trees where the intermediate is also Reeves', while the growth of the Oullins trees is increased by all intermediates compared with trees where the top scion and intermediate were both Oullins.

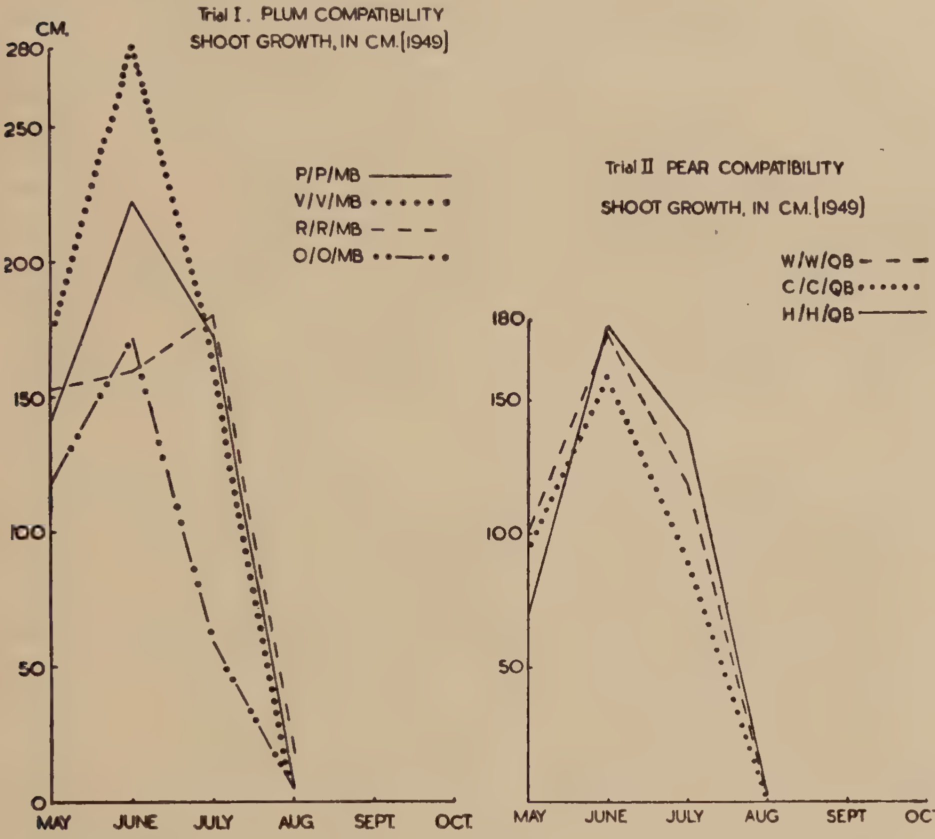


FIG. 7.

Monthly increment in shoot growth.

Some of these effects of the intermediate on tree growth may be brought about by an alteration in growth rate at one particular time of the year. This point may be appreciated for some of the plum combinations by comparing Figs. 6 and 8 and Table I. The rapid falling off in the growth of Victoria on Myrobalan B (V/V/MB) in July can be seen from Fig. 6 while the same figure also shows that the growth rate of President on Myrobalan B (P/P/MB) fell off much less during that month ; Table I shows that the combination V/P/MB made more growth in height during 1948 than V/V/MB although President on Myrobalan B (P/P/MB) was less vigorous than Victoria (V/V/MB) ; this effect may be explained by reference to Fig. 8 which shows that the greater size of V/P/MB was due to a considerable increase in growth rate in

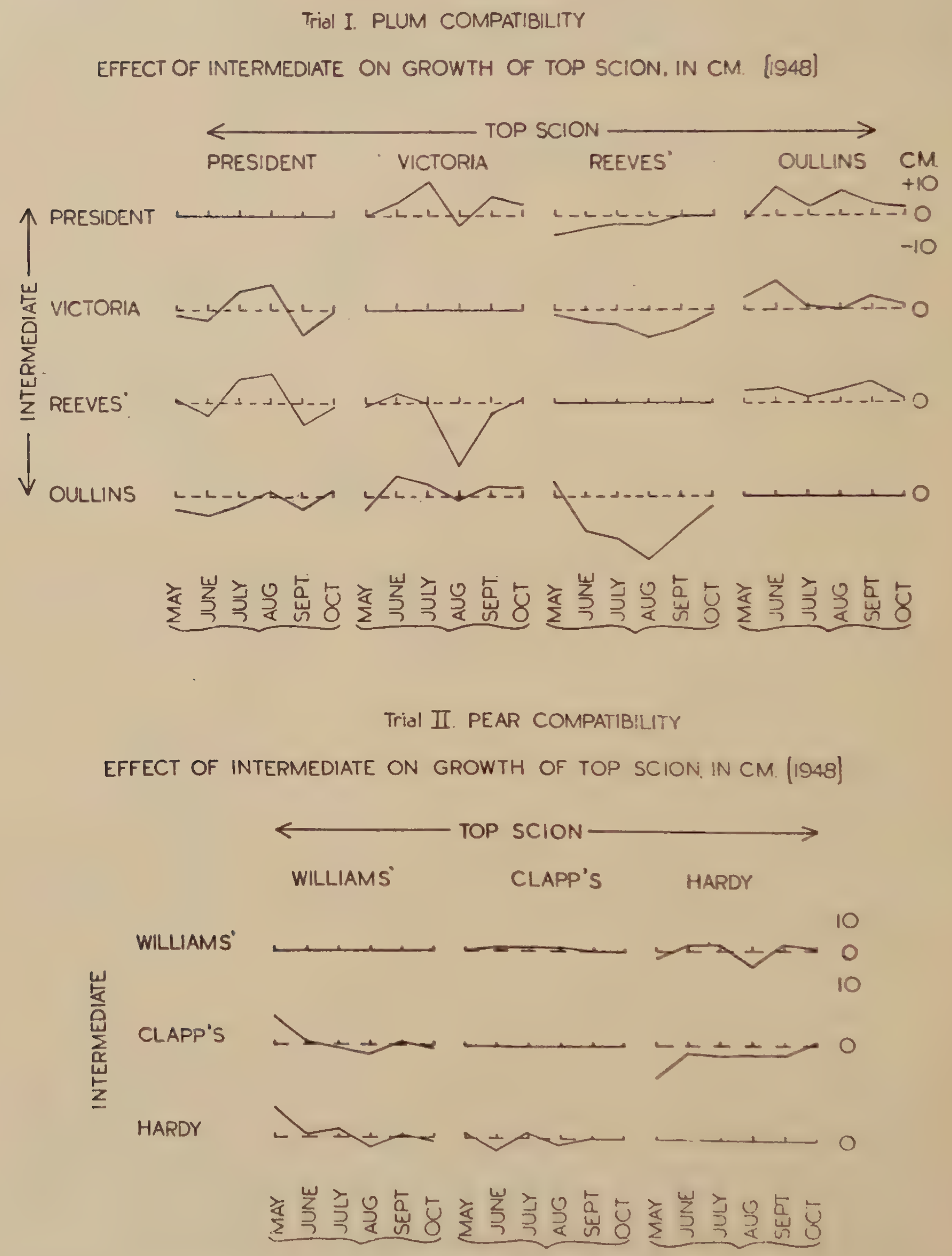


Fig. 8.

Diagrams to illustrate the effect of the intermediate on the monthly increment in shoot growth.

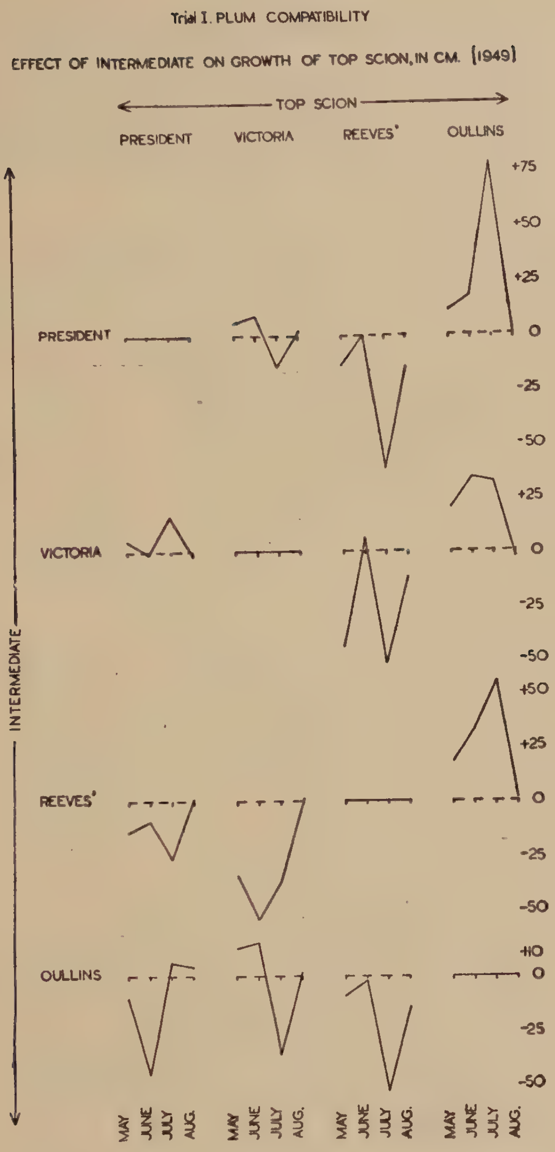


FIG. 9.

Diagram to illustrate the effect of the intermediate on the monthly increment in shoot growth.

July probably due to the presence of the President intermediate. In considering this effect it is of interest to note that some workers (Elliott, 1933 and 1935 ; Gill, 1932 and Priestley and Scott, 1936) consider that the relative rate of production of xylem and phloem is correlated with shoot elongation, i.e. during the period when the leaves are expanding, the cambium is cutting off a higher proportion of xylem to phloem than it is when growth in length of shoot is proceeding slowly, or not at all. The relative amounts of xylem and phloem have been shown to be correlated with rootstock effects on vigour by Beakbane (1941) and Beakbane and Thompson (1947) and it seems possible therefore that differences in period of growth of the components of the grafted tree could be contributory factors in the resulting vigour of the tree.

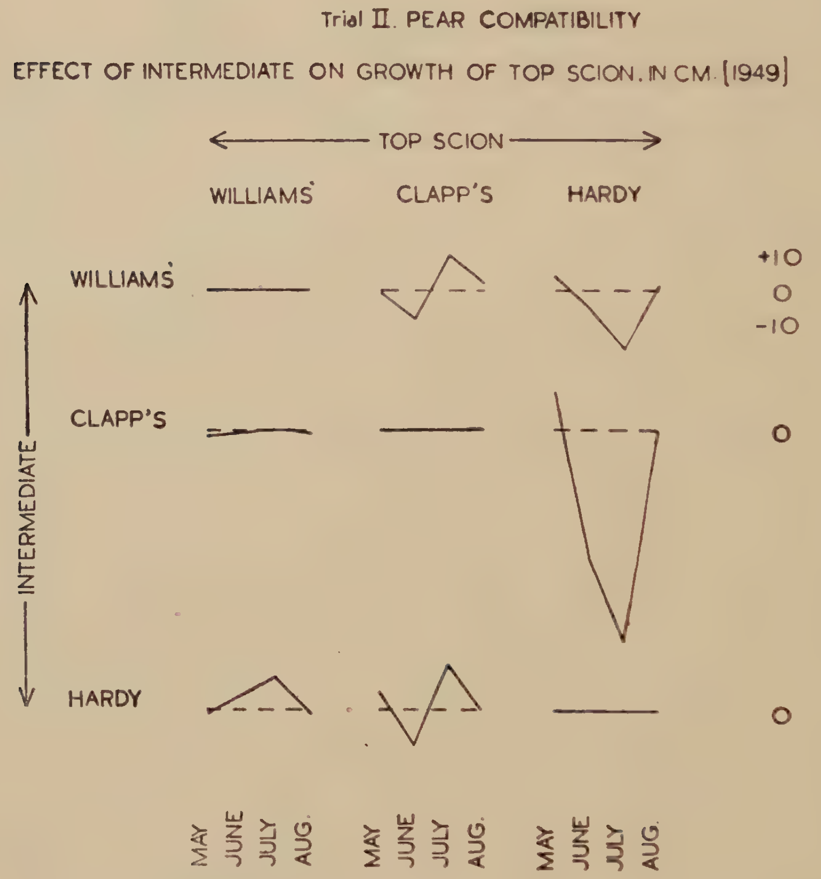


FIG. 10.

Diagram to illustrate the effect of the intermediate on the monthly increment in the shoot growth.

(*b*) *Girth increment.*

In 1948 the two periods of maximum increase in the girth of the rootstock stem coincided with the time of maximum shoot elongation, viz. June and August (Tables III, IV and Fig. 6). The tallest trees also had the greatest girth increment.

TABLE III.

Trial I. Plum compatibility.

Monthly girth increment of the rootstock, 1948 (mm.).

Combination.	Mean of twelve trees.					
	June.	July.	August.	September.	October.	Total.
P/P/MB	3	3	6	4	1	17
P/V/MB	3	3	5	4	1	16
P/R/MB	3	2	6	3	1	15
P/O/MB	3	3	5	4	1	16
V/P/MB	5	4	5	4	0	18
V/V/MB	4	3	5	4	1	17
V/R/MB	4	3	5	3	1	16
V/O/MB	3	5	6	5	1	20
R/P/MB	3	2	3	3	1	12
R/V/MB	4	3	3	3	0	13
R/R/MB	3	3	4	3	1	14
R/O/MB	3	2	2	3	0	10
O/P/MB	3	2	4	4	0	13
O/V/MB	4	2	4	3	1	14
O/R/MB	4	1	3	3	1	12
O/O/MB	3	1	3	4	1	12
Total ..	55	42	69	57	12	

TABLE IV.

Trial II. Pear compatibility.

Monthly girth increment of the rootstock, 1948 (mm.).

Combination.	Mean of eighteen trees.					
	June.	July.	August.	September.	October.	Total.
W/W/QB	4	1	3	2	1	11
W/C/QB	4	2	3	2	1	12
W/H/QB	4	2	3	3	1	13
C/W/QB	4	2	3	2	0	11
C/C/QB	4	2	3	1	1	11
C/H/QB	4	2	3	2	0	11
H/W/QB	4	2	4	2	1	13
H/C/QB	2	2	3	2	1	10
H/H/QB	4	1	4	4	1	14
Total ..	34	16	29	20	7	

TABLE V

Trial I. Plum compatibility.

Girth increment of top scion, intermediate and rootstock, 1949 (mm.).

Combination.	Root-stock.	Inter-mediate.	Top-scion.	Combination.	Root-stock.	Inter-mediate.	Top-scion.
P/P/MB (8)	23	26	23	R/P/MB (9)	20	27	28
P/V/MB (7)	18	20	23	R/V/MB (6)	16	18	24
P/R/MB (7)	24	29	25	R/R/MB (5)	24	31	33
P/O/MB (9)	19	24	25	R/O/MB (8)	17	22	23
V/P/MB (8)	21	25	19	O/P/MB (7)	22	26	25
V/V/MB (8)	19	20	20	O/V/MB (7)	13	15	16
V/R/MB (5)	21	22	19	O/R/MB (5)	17	20	18
V/O/MB (8)	19	22	19	O/O/MB (7)	17	19	18

TABLE VI.

Trial II. Pear compatibility.

Girth increment of top scion, intermediate and rootstock, 1949 (mm.).

Combination.	Root-stock.	Inter-mediate.	Top-scion.	Combination.	Root stock.	Inter-mediate.	Top-scion.
W/W/QB (12)	7	10	12	C/H/QB (13)	9	11	8
W/C/QB (14)	6	9	10	H/W/QB (14)	6	10	12
W/H/QB (14)	9	12	9	H/C/QB (14)	5	9	10
C/W/QB (14)	5	8	9	H/H/QB (14)	11	14	13
C/C/QB (14)	4	8	9				

NOTE.—The figures in brackets after each combination represent the number of trees of which means were taken.

The girth increment of the different components of the stem during 1949 is shown in Tables V and VI. It is interesting to note that the girth of the rootstock was greater under the compatible intermediate Hardy than under the incompatible varieties Clapp's and Williams', while in the plum trial the girth of the rootstock was greatest under one compatible and one incompatible intermediate, viz. President and Reeves'.

(4) DEFOLIATION.

In both compatible and incompatible combinations the autumn defoliation began in a normal way at the base of the shoots and proceeded upwards. In this respect these trees differed from those of Chang (1938), since the defoliation in the incompatible combinations included in his trials began from the top of the shoots. The data for autumn defoliation given in Table I show that in the plum and pear trials, the defoliation was a varietal characteristic depending on the top scion.

Chang (1938) suggested, that the differences in period of cambial activity between stock and scion may be responsible for the accumulation of inert functionless tissue at the union, as a constant breaking and " re-grafting " of the uniting cells takes place.

(5) INITIATION OF CAMBIAL ACTIVITY.

Several workers have shown that in many species cambial activity begins in spring in the opening buds and thence spreads basipetally (Swarbrick, 1927; Sledge, 1930; Knight, 1930 and Priestley, 1933). In the hardwoods, the basipetal differentiation of new wood takes place at somewhat different times relative to the degree of opening of the buds, and at very different rates, in different species (Priestley, 1933).

The following observations were made by the strip method described above:

(1) In 1948 cambial activity, as judged by the capacity to yield strips, started below each opening bud during the second fortnight in April.

(2) The spreading of cambial activity to the internodes took place only when extension growth began about 10-15 days after activity could be observed below the buds.

(3) When cambial activity spread basipetally there was no evidence that it was held up by any of the unions, either in compatible or incompatible combinations. On the contrary, cambial activity began in the intermediate and rootstock in a normal basipetal way.

When cambial activity began in the rootstock Myrobalan B in Trial I, observations were made on some adjacent ungrafted Myrobalan B rootstocks. The ungrafted Myrobalan B plants were found to be in a more advanced stage of cambial activity. It appears, therefore, that cambial activity in the rootstocks in Trial I only began when the stimulus from the scions reached the rootstock stem. This is perfectly understandable as the rootstock had no buds of its own that could have induced cambial activity beforehand and it is reasonable to suppose that the stimulus travelling down from the top-scion activated the cambium of the intermediate and of the rootstock. The fact that the stimulus was normally transmitted through incompatible unions is not surprising. Snow (1932) found that the cambial stimulus " . . . can pass across a protoplasmic discontinuity and even through an interposed piece of moist linen. . . . ."

(6) GENERAL APPEARANCE OF TREES.

During the first year's growth the trees did not exhibit any external symptoms of ill-health.

During the second year's growth many of the trees in Trial I showed signs of lack of water. Both compatible and incompatible combinations showed these drought symptoms, trees with Victoria and Oullins as top-scions being the most affected. In Trial II, about 25 per cent. of the trees in the combination H/W/QB and 50 per cent. in the combination H/C/QB developed reddish colour in their foliage during August, 1948. Although the presence of red colour in pear leaves is not necessarily a symptom of ill-health, the fact that none of the trees H/H/QB showed reddish colour probably indicates that the combinations H/C/QB and H/W/QB suffered from some disability due to the intermediate.

HISTOLOGICAL STRUCTURE.

Relative area of vessels, fibres, parenchyma cells and rays in the wood.

For the plum trial the only trees examined for the relative area of the four elements in the wood were those where the top-scion and intermediate were of the same variety and they may, therefore be regarded as being similar to single-worked trees except for the presence of a second union. In the pear trial all combinations were examined for these characteristics.

(*a*) *Vessels.* The relative area of vessels in the plum scions Victoria and President was greater than in the stem of the rootstock Myrobalan B as shown in Table VII where the differences of the means of the transformed percentages (see p. 193) are given in the last two columns. The differences are only significant at the 5 per cent. level if they are greater than the figure given for the significant difference at the base of the column.

TABLE VII.

Trial I. Plum compatibility.

Relative area of elements in the wood.

Column.		1.	2.	3.	4.	5.	6.
		Percentage area.				Diff. of means for θ.	
Type of element.	Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Stock-root Sect. IV.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
Vessels	P/P/MB	12·0	14·6	6·8	14·6	−2·10	+6·15
	V/V/MB	22·8	20·8	13·5	11·6	+1·35	+6·62
	R/R/MB	15·8	17·8	15·6	9·3	−1·50	+0·95
	O/O/MB	15·1	10·6	13·9	11·7	+3·85	−1·02
	Sig. diff.					5·34	4·63
Fibres	P/P/MB	67·4	70·7	62·5	34·5	−2·00	+4·00
	V/V/MB	53·7	57·7	55·5	33·3	−2·30	+0·15
	R/R/MB	64·9	56·0	60·7	40·7	−5·25	−0·13
	O/O/MB	64·3	69·5	66·2	35·1	+3·10	+0·45
	Sig. diff.					3·14	2·72
Parenchyma	P/P/MB	0·8	0·3	1·8	18·1	+3·10	−3·85
	V/V/MB	1·6	0·9	2·1	22·3	+1·90	−2·00
	R/R/MB	0·5	0·8	2·9	22·8	−1·00	−5·35
	O/O/MB	1·6	0·9	1·7	21·7	+1·55	−1·85
	Sig. diff.					3·33	2·88
Rays	P/P/MB	19·7	14·4	29·0	33·0	+4·15	−8·22
	V/V/MB	21·7	20·5	28·8	32·8	+0·85	−5·07
	R/R/MB	18·7	25·4	20·7	27·2	−4·50	+0·85
	O/O/MB	18·9	18·9	18·1	31·5	0·00	+0·70
	Sig. diff.					5·42	4·69

Level of significance P = 0·05.

In general no difference in relative vessel area occurred between the stems of the same variety growing as top-scion and intermediate in plums (Table VII, Col. 5) or in pears (Table VIII, Col. 5). There was a difference, however, between Williams' and Hardy in the combination W/H/QB (Table VIII, Col. 5).

There were numerous differences in vessel area between rootstock and intermediate in pears. Quince B had a smaller area of vessels than the following intermediates, (1) Williams' in the combination H/W/QB, (2) Hardy in C/H/QB and H/H/QB and (3) Clapps' in H/C/QB, W/C/QB and C/C/QB (Table VIII, Col. 6).

TABLE VIII.

Trial II. Pear compatibility.

Relative area of vessels.

Column.	1.	2.	3.	4.	5.	6.
	Percentage area.				Diff. of means for θ.	
Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Stock-root Sect. IV.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
W/W/QB	23·0	15·7	13·9	15·8	+3·08	+2·46
W/C/QB	20·1	24·2	12·1	11·2	−3·68	+9·11
W/H/QB	25·7	18·4	16·3	11·6	+5·01	+1·56
C/W/QB	27·3	21·5	17·0	12·6	+3·88	+3·31
C/C/QB	19·9	19·8	13·0	10·7	+0·13	+5·20
C/H/QB	22·8	19·6	13·2	12·4	+2·16	+4·95
H/W/QB	22·9	23·8	13·8	11·5	−0·58	+7·42
H/C/QB	31·7	27·6	15·8	13·4	+2·61	+8·29
H/H/QB	24·4	21·7	13·0	9·0	+1·79	+6·91
			Sig. diff.	(P = 0·05)	..	4·93

(*b*) *Fibres.* President was the only plum variety with a larger percentage area of fibres than Myrobalan B (Table VII, Col. 6). The difference between top-scion and intermediate, Section I and II, in relative area of fibres for the variety Reeves' (Table VII, Cols. 1, 2 and 5) is the only case in which a significant difference was found between sections of the same variety. A close examination of the sections showed that although both Sections I and II contained much " tension wood "* it was more fully developed in the middle of the annual ring of Section I, where the measurements were made. This fact could account for the difference as it is known that the proportion of fibres is normally greater in " tension " than in ordinary wood.

In the pear trial there were no differences in percentage area of fibres either within the varieties or between the varieties and the rootstock (Table IX, Cols. 5 and 6).

(*c*) *Parenchyma cells.* The plum varieties President and Reeves' had a smaller percentage area of parenchyma cells in the wood than Myrobalan B (Table VII, Col. 6). All the pear varieties used as intermediates had a smaller percentage area of parenchyma cells than the Quince B rootstock (Table X, Col. 6). The varieties Williams' and Clapp's also differed in amount of parenchyma cells in the combination W/C/QB (Table X, Col. 5).

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* Tension wood is a specialized type of wood formed as a result of certain stresses set up in growing trees. It is frequently found in forest trees and is described by S. H. Clarke (1939) in Special Report No. 5 of the Forest Products Research Laboratory. The same type of wood is also found in young plum trees.

TABLE IX.

Trial II. Pear compatibility.

Relative area of wood fibres.

Column.	1.	2.	3.	4.	5.	6.
	Percentage area.				Diff. of means for θ.	
Combination.	Top scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Stock-root Sect. IV.	Sect. I. minus Sect. II.	Sect. I & II minus Sect. III.
W/W/QB	45·6	51·7	44·0	27·3	−4·76	+4·30
W/C/QB	40·1	48·7	50·2	26·4	−5·10	−0·82
W/H/QB	47·8	50·7	44·9	24·3	−1·92	+3·33
C/W/QB	46·4	47·3	45·6	25·2	−0·34	+0·72
C/C/QB	50·9	49·3	46·3	26·7	+0·51	+3·16
C/H/QB	43·7	48·2	50·1	22·2	−2·64	−1·11
H/W/QB	50·5	47·5	46·7	25·1	+1·73	+0·42
H/C/QB	48·7	44·5	45·7	22·6	+2·41	+0·72
H/H/QB	51·0	51·0	50·5	29·1	−0·03	+0·31
			Sig. diff.	(P = 0·05)	..	5·40

TABLE X.

Trial II. Pear compatibility.

Relative area of wood parenchyma.

Column.	1.	2.	3.	4.	5.	6.
	Percentage area.				Diffs. of means for θ.	
Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Stock-root Sect. IV.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
W/W/QB	14·4	18·6	26·9	38·5	−1·05	− 8·84
W/C/QB	21·0	9·0	22·0	48·9	+9·36	−10·98
W/H/QB	12·0	12·4	20·9	48·3	−0·20	− 6·67
C/W/QB	11·1	12·3	21·4	46·8	−1·04	− 7·01
C/C/QB	11·8	13·6	23·5	47·9	−1·59	− 7·36
C/H/QB	14·8	10·4	23·6	43·3	+3·84	−10·52
H/W/QB	9·1	12·5	22·7	47·7	−3·19	− 7·90
H/C/QB	7·7	8·8	21·4	46·3	−0·99	−10·39
H/H/QB	10·5	9·1	21·7	45·6	+1·09	−10·17
			Sig. diff.	(P = 0·05)	..	6·22

(*d*) *Rays.* The relative area of rays was greater in Myrobalan B than in the scions Victoria and President (Table VII, Col. 6), while in pears the amount of ray tissue was very similar in rootstock and scion except between Hardy and Quince B in the combination C/H/QB (Table XI, Col. 6).

TABLE XI.

Trial II. Pear compatibility.
Relative area of wood rays.

Column.	1.	2.	3.	4.	5.	6.
	Percentage area.				Diff. of means for θ.	
Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Stock-root Sect. IV.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
W/W/QB	16·8	13·9	15·2	18·2	+3·08	+2·46
W/C/QB	18·8	17·9	15·7	13·4	+0·06	+1·73
W/H/QB	14·4	18·3	17·8	15·6	−2·97	−0·31
C/W/QB	15·2	18·8	16·0	15·4	−2·72	+2·11
C/C/QB	17·4	17·1	17·2	14·6	+0·18	+0·10
C/H/QB	18·6	21·8	13·0	22·0	−2·36	+6·67
H/W/QB	17·6	16·1	16·7	15·5	+1·13	+0·48
H/C/QB	11·7	19·0	17·2	17·5	−5·84	−1·50
H/H/QB	14·1	18·1	14·7	16·3	−2·93	+2·39
			Sig. diff.	(P = 0·05)	.. 5·93	

TABLE XII.

Trial I. Plum compatibility.
Size of elements in the wood.

Column.		1.	2.	3.	4.	5.
		Sq. μ.			Diff. of means.	
Type of element.	Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
Vessels ..	P/P/MB	1272	1090	935	+182	+246
	V/V/MB	1495	1358	968	+137	+459
	R/R/MB	1474	1209	1198	+265	+144
	O/O/MB	995	1084	979	− 89	+ 60
	Sig. diff.				357	309
Fibres ..	P/P/MB	148	142	94	+ 6	+ 51
	V/V/MB	140	141	108	− 1	+ 32
	R/R/MB	188	157	92	+ 31	+ 81
	O/O/MB	141	146	103	− 5	+ 41
	Sig. diff.				329	28
Parenchyma	P/P/MB	118	145	129	− 27	+ 2
	V/V/MB	170	162	127	+ 8	+ 39
	R/R/MB	188	179	118	+ 9	+ 65
	O/O/MB	195	184	140	+ 11	+ 49
	Sig. diff.				60	52

Level of Significance P = 0·05.

Size of vessels, fibres and parenchyma cells.

(*a*) *Vessels.* The vessel size differed from that of the roostock in one plum variety, the compatible Victoria, and one pear variety, the incompatible Clapp's, the vessels being larger than the rootstock in Victoria and smaller in Clapp's (Table XII, Col. 5 and Table XIII, Col. 5). Fig. 11 illustrates the similarity of vessel area and vessel size for the incompatible varieties Oullins and Myrobalan B, and the difference that occurred in these characteristics between the compatible varieties Victoria and Myrobalan B.

TABLE XIII.

Trial II. Pear compatibility.
Size of elements in the wood.

Column.		1.	2.	3.	4.	5.
		Sq. μ.			Diff. of means.	
Type of element.	Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
Vessels ..	W/W/QB	1038	1073	957	− 35	+ 98
	C/C/QB	861	1003	1119	−142	−187
	H/H/QB	1200	1208	1299	− 8	− 95
	Sig. diff.				164·0	141·9
Fibres ..	W/W/QB	233	208	196	+ 15	+ 19
	C/C/QB	170	183	187	− 13	− 11
	H/H/QB	197	247	210	− 50	+ 12
	Sig. diff.				70·1	60·7
Parenchyma	W/W/QB	249	266	270	− 17	− 13
	C/C/QB	203	220	258	− 17	− 47
	H/H/QB	244	232	271	+ 12	− 33
	Sig. diff.				26·4	23

Level of significance P = 0·05.

(*b*) *Fibres.* The fibres of all the plum varieties were larger than those of the rootstock Myrobalan B (Table XII, Col. 5). No differences occurred in size of fibres between the pear varieties and the Quince B rootstock (Table XIII, Col. 5), or between top scion and intermediate in either pears or plums.

(*c*) *Parenchyma cells.* The parenchyma cells of the incompatible Reeves' were larger than those of Myrobalan B, while in the pears one compatible and one incompatible variety, viz. Hardy and Clapp's, had smaller parenchyma cells than Quince B (Table XII, Col. 5 and Table XIII, Col. 5).

Measurement of the rays in the cambial zone.

The width of the ray initial in the cambial zone for uniseriate rays is given for plums in Table XIV and for pears in Table XV. The ray initials were larger in the scion than in the rootstock for the combinations President/Myrobalan B, Reeves'/Myrobalan B and Clapp's/Quince B.

The distance between the rays in the cambial zone was greater in every plum variety than in the rootstock, while there was no difference between the pear varieties and the Quince B rootstock in this respect. (Tables XIV, XV, Col. 5).

TABLE XIV.

Trial I. Plum compatibility.

Distance between rays, and width of ray initial for uniseriate rays.

Column.		1.	2.	3.	4.	5.
		μ			Diff. of means.	
	Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
Distance between rays	P/P/MB	42·5	50·6	38·7	− 8·1	+ 7·8
	V/V/MB	62·0	61·0	49·6	+ 1·0	+11·9
	R/R/MB	63·5	58·7	47·4	+ 4·8	+13·7
	O/O/MB	52·9	57·4	46·6	− 4·5	+ 8·5
	Sig. diff.				8·14	7·05
Width of ray initial in uniseriate rays	P/P/MB	12·9	12·8	10·7	+ 0·1	+ 2·2
	V/V/MB	11·8	12·2	11·0	− 0·4	+ 1·0
	R/R/MB	12·3	12·3	10·0	0·0	+ 2·3
	O/O/MB	11·5	11·8	10·2	− 0·3	+ 1·5
	Sig. diff.				2·03	1·75

Level of significance P = 0·05.

TABLE XV.

Trial II. Pear compatibility.

Distance between rays, and width of ray initial for uniseriate rays.

Column.		1.	2.	3.	4.	5.
		μ			Diff. of means.	
	Combination.	Top-scion Sect. I.	Intermediate Sect. II.	Stock-stem Sect. III.	Sect. I minus Sect. II.	Sect. I & II minus Sect. III.
Distance between rays	W/W/QB	53·1	50·9	50·9	+ 2·2	− 1·9
	C/C/QB	54·7	49·1	48·6	+ 5·6	+ 8·3
	H/H/QB	51·2	49·6	52·8	+ 1·6	− 2·4
	Sig. diff.				16·9	14·6
Width of uniseriate ray initial	W/W/QB	9·7	9·9	9·0	− 0·2	+ 0·8
	C/C/QB	10·3	10·4	8·5	− 0·1	+ 1·8
	H/H/QB	10·7	10·3	9·5	+ 0·4	+ 1·0
	Sig. diff.				1·64	1·41

Level of significance P = 0·05.

Influence of the scion on the structure of the stock.

The question of whether the structure of either the scion or the stock in the grafted tree is affected by the other part is a point of interest. Beakbane (1941) found that some histological characteristics of the apple variety Lane's Prince Albert were not affected when grafted on three different Malling rootstocks. In the present trial the possible effect of different scion varieties on the histological structure of the two rootstocks, Myrobalan B and Quince B was examined. The percentage of the four elements in the wood of the rootstock under different varieties has already been expressed for the stem (Section III) and for the root (Section IV) in Tables VII to XI. An analysis of variance was carried out for the relative area of vessels, fibres, parenchyma cells and rays in the stems and roots of the rootstock under various scions, In no case was there a significant effect of scions.

In comparing the structure of the top-scion, intermediate and rootstock one should bear in mind the point that in a normal ungrafted tree a certain gradient down the stem in size and proportion of elements would exist, and that such a gradient would also be necessary for equilibrium in a grafted tree. Weinstein (1926) points out that such a gradient exists for the wood rays in some trees and Rendle *et al.* (1934) discusses the variation in the size of elements in relation to the position in height on the stem. The gradients indicated by the data for various elements in the transverse sections I, II, III give no indications of being different for the compatible and incompatible combinations, although it should be borne in mind that the length of stem covered by these sections is very short compared with the total length of the tree.

Differences in histological structure between varieties grafted together were observed in many combinations, but no correlation was found between the occurrence of such differences and the degree of compatibility, and there was no indication that the internal structures of the stock and scion were more similar for the compatible than for the incompatible combinations in the characteristics examined. The compatible plants Victoria and Myrobalan B differed significantly in five out of nine histological characteristics studied while the incompatible plants Oullins and Myrobalan B differed only in two. Some of the characteristics seemed to be linked to botanical groups since all the plum varieties (*Prunus domestica*) had larger fibres and a greater distance between rays than Myrobalan B (a clone of *Prunus cerasifera*), and all the pear varieties (*Pyrus communis*) had a smaller percentage area of parenchyma than Quince B (a clone of *Cydonia oblonga*). Other histological characteristics, such as the vessel area, varied greatly between stock and scion but did not appear to be associated with incompatibility since the variations occurred in both compatible and incompatible combinations.

INTERNAL EXAMINATION OF THE UNIONS.

Macroscopical observations.

Although none of the young trees included in these trials showed external symptoms of incompatibility, the internal examination of the unions revealed in many of them, conditions symptomatic of such a disorder.

In order to get a picture of the construction of the unions, in the four trees of each combination examined in autumn, 1949, two kinds of observations were made : (1) on the macroscopical appearance of a radial longitudinal section of the union showing the relative positions of the original cut surfaces of the graft and the degree

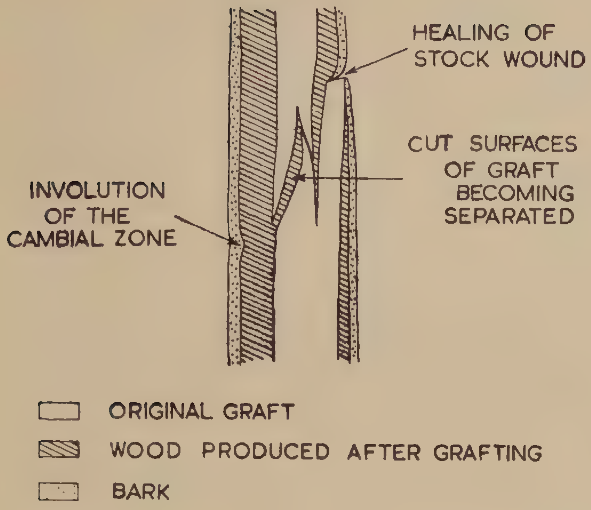
HEALING OF STOCK WOUND
CUT SURFACES OF GRAFT BECOMING SEPARATED
INVOLUTION OF THE CAMBIAL ZONE
▭ ORIGINAL GRAFT
▨ WOOD PRODUCED AFTER GRAFTING
⬚ BARK

FIG. 12.

Diagram of radial longitudial section of a graft union illustrating some of the defects in the structure described in the text.

of union between the tissues formed by stock and scion since grafting, and (2) on the external appearance of the stem at the union after the bark had been peeled off, which showed the degree of continuity of the wood at the end of the second growing season.

The characteristics recorded at the line of union were as follows :

(1) the presence of parenchymatous tissue in the wood, either as isolated areas or as a continuous layer.
(2) discontinuity in the bark, as indicated by a layer of brown cells, probably cork.
(3) involution of the cambial zone, resulting from a local decrease in radial growth at the point of union (Fig. 12).
(4) the degree of healing of the stock wound opposite the base of the scion (Fig. 12).
(5) separation of the cut surfaces of the original graft by a layer of lignified callus or ingrowing bark (Fig. 12).

Characteristics, (1) parenchymatous tissue in the wood, and (2) discontinuity in the bark, are generally considered to be symptomatic of incompatibility (Argles, 1937 ; Chang, 1938). In the plum unions discontinuity in the wood was the main symptom of incompatibility, and discontinuity in the bark was seldom recorded. In the incompatible pear-quince unions discontinuity in the bark was the main feature; discontinuous wood appearing in general, only at the end of the second year's growth. This difference between the plum and the pear/quince unions has been previously recorded in some combinations (Chang, 1938).

Characteristic (3) involution of the cambial zone, is not in itself a symptom of incompatibility (Bradford and Sitton, 1929) although it may precede a more definite discontinuity in the wood.

Characteristic (4) the degree of healing of the stock wound, depended, for the young trees examined, rather on the relative diameters of stock and scion than on incompatibility.

Finally characteristic (5) cut surfaces of graft becoming separated, was frequently associated with (1) parenchymatous tissue in wood, and (2) discontinuity in bark.

Attaching most importance to points 1 and 2, four categories have been established for the classification of unions in each combination as follows :

Category A. The four unions examined showed continuous bark and wood.

Category B. Some of the unions examined showed some structural defects possibly associated with incompatibility, such as slight discontinuity, or distortion in the wood or bark, or involution of the cambium at the line of union. It is likely that some of the unions included in this category would have developed definite macroscopical symptoms of incompatibility in later years.

Category C. Some of the unions examined showed evidence of either a layer of parenchymatous tissue in the wood and/or discontinuity in the bark at the line of union.

Category D. All the unions examined showed one of the conditions described in category C.

Tables XVI and XVII give the category for each group of unions in Trials I and II respectively.

TABLE XVI.

Trial I. Plum compatibility.

Classification of unions on their macroscopical appearance.

Combination.	Union.	Category.	Combination.	Union.	Category.
P/P/MB	P/P	A	R/P/MB	R/P	A
	P/MB	B		P/MB	B
P/V/MB	P/V	B	R/V/MB	R/V	C
	V/MB	B		V/MB	B
P/R/MB	P/R	B	R/R/MB	R/R	B
	R/MB	C		R/MB	D
P/O/MB	P/O	A	R/O/MB	R/O	C
	O/MB	C		O/MB	D
V/P/MB	V/P	C	O/P/MB	O/P	B
	P/MB	C		P/MB	B
V/V/MB	V/V	A	O/V/MB	O/V	A
	V/MB	B		V/MB	B
V/R/MB	V/R	C	O/R/MB	O/R	C
	R/MB	D		R/MB	D
V/O/MB	V/O	A	O/O/MB	O/O	A
	O/MB	C		O/MB	D

A. All four unions normal.
B. Some unions with slight structural defects.
C. Some unions with layer of parenchymatous tissue in wood and/or discontinuity in bark.
D. All four unions with layer of parenchymatous tissue in wood and/or discontinuity in bark.

TABLE XVII.

Trial II. Pear compatibility.

Classification of unions on their macroscopical appearance.

Combination.	Union.	Category.	Combination.	Union.	Category.
W/W/QB	W/W	B	C/H/QB	C/H	A
	W/QB	D		H/QB	B
W/C/QB	W/C	A	H/W/QB	H/W	A
	C/QB	D		W/QB	D
W/H/QB	W/H	B	H/C/QB	H/C	B
	H/QB	B		C/QB	D
C/W/QB	C/W	A	H/H/QB	H/H	A
	W/QB	D		H/QB	A
C/C/QB	C/C	B			
	C/QB	D			

A. All four unions normal.
B. Some unions with slight structural defects.
C. Some unions with layer of parenchymatous tissue in wood and/or discontinuity in bark.
D. All four unions with layer of parenchymatous tissue in wood and/or discontinuity in bark.

Unions showing symptoms of incompatibility were mechanically strong with the exception of a few unions between intermediate and stock in the plum trial.

Variability. The structure of the union in plums (Trial I) appeared to show considerable variability.

In the first place, there was variability within the union. As revealed by the peeling off of the bark, discontinuity in the wood was present in some places, while in others bridges of perfectly connected wood elements of stock and scion had been established. Fig. 13 shows some of this bridging tissue alternating with areas previously filled with parenchymatous tissue in a union between Victoria and Reeves'.

Secondly, there was variability within the four trees of each combination especially in the plum unions. In view of the fact that the trees were grown under comparable conditions, it is surprising that such symptoms of incompatibility as could be seen macroscopically were sometimes recorded for only one or two trees out of the four examined. It seems likely, however, that if it had been possible to obtain a picture of the complete surface of the union, instead of just the part exposed to view in a radial longitudinal section, this apparent variability would have been reduced. Moreover the structure of the unions as seen with the aid of a microscope proved to be more consistent within each group and more clearly related to the compatibility of the graft combinations than the appearance of the unions to the naked eye. The microscopical structure is described below.

Incompatibilities between top-scion and intermediate. The following combinations showed macroscopical symptoms of incompatibility at the union of top-scion and intermediate, in at least one of the four unions examined.

(1) Oullins on Reeves' and the reciprocal Reeves' on Oullins.

(2) Victoria on Reeves' and the reciprocal Reeves' on Victoria. Reeves' was the most damaged part in the association with Victoria as shown by dead zones in the older wood and areas of parenchymatous tissue located mainly on the Reeves' side (Fig. 13).

(3) Victoria on President in the combination V/P/MB. This case is discussed below.

Incompatibilities between intermediate and rootstock and the influence of the top-scion variety on the lower union. The varieties Reeves', Oullins, Williams' and Clapp's, known to be incompatible when single-grafted on the rootstocks used in these trials, manifested symptoms of incompatibility at the union with the rootstock when they were used as intermediates. For the pear varieties, Williams' and Clapp's, these symptoms were not reduced by the use of a top-scion compatible with the rootstock. On the other hand, it seems that President and Victoria as top-scions improved some of the unions between Oullins (intermediate) and Myrobalan B, and President as top-scion improved some of the unions between Reeves' (intermediate) and Myrobalan B (Tables XVI and XVII). This fact suggests that there may be some influence of the top-scion variety on the union between intermediate and rootstock, though not enough to overcome their incompatibility. Incompatibility, in these trees, seems to be due to a reaction of the tissues at the lower union rather than to the leaves of the top-scion variety.

The union between Hardy as intermediate and the rootstock Quince B was perfectly compatible in the combination H/H/QB and this compatibility was not greatly changed by the use of a top-scion variety incompatible with the rootstock. The fact that the H/QB unions in the combinations C/H/QB and W/H/QB sometimes showed defects not present in the combination H/H/QB suggests, again, some kind of influence of the top-scion variety on the union between intermediate and rootstock modifying though not completely changing their compatibility.

Finally, it must be pointed out that the unions between Victoria and President and the rootstock Myrobalan B in the combinations V/V/MB and P/P/MB showed slight structural defects at the union that could be interpreted as symptomatic of incompatibility. As the combinations V/MB, and P/MB are growing satisfactorily in orchards, one must conclude that such slight defects at the union will not develop sufficiently to affect the behaviour of the tree to any great extent. On the other hand, they may help to explain the more definite symptoms of incompatibility found at the union P/MB in the combination V/P/MB.

Combination V/P/MB. The incompatibility of this combination was first observed by Montgomery, Moore and Hoblyn (1943). The trees were raised by budding President on rooted layers of Myrobalan B in 1932 and by top-grafting Victoria in 1935 on each of the main branches formed above the crotch. Five years later (1940) many of the trees showed external symptoms of incompatibility and by 1946 a few trees had died. Roach and Thompson (1947) studied one of the trees which showed signs of incompatibility in one of its two branches to a greater degree than in the other. The top union (V/P) appeared to be perfect while in the lower union P/MB the wood was discontinuous and the beginning of the discontinuity corresponded with the removal of all the President foliage.

In the present experiment slight symptoms of incompatibility were found in both top and lower unions of some trees of such combinations, and it is probable that (1) the age of the trees, (2) the different way in which the trees were built up and (3) the lack of leaves on the intermediate may have accounted for the different results.

No complete explanation for the incompatibility of this combination can be offered but it seems that President on Myrobalan B, although producing satisfactory trees for fruit production, has some kind of mild incompatibility that could increase under certain circumstances, as for instance, when top-grafted with Victoria. The slight incompatibility between Victoria and President may tend to increase the incompatibility in the lower union. That President and Myrobalan B are not fully compatible is illustrated in Fig. 14 which shows a photomicrograph of the lower union of a one-year-old tree P/P/MB showing signs of incompatibility.

Differences in oxidation of the tissues of rootstock and scion. When the rootstock and scion wood were exposed to the air at the time that the bark was peeled from the unions, differences in colour due to oxidation of the tissues were observed. The change from light to dark brown frequently occurred suddenly at the line of union showing that rootstock and scion retained their own biochemical constitutions in this respect.

Quality of unions and growth. Attempts to correlate the macroscopical appearance of the unions with the separate records of growth for each tree within each combination, gave no positive results. Within the trees of one combination it was surprising to find that more pronounced macroscopical symptoms of incompatibility at the union sometimes appeared in the more vigorously growing trees rather than in the weaker ones. This probably indicates that the rate of growth may influence the appearance of incompatibility symptoms at the union.

An incidental observation on the cambial activity was made when peeling the bark of the two-year-old unions in October. A more advanced state of dormancy seemed to exist below the discontinuous areas at the union. In compatible unions no difference in activity was recorded above and below the unions, and therefore the earlier dormancy of the Quince B cannot be regarded as a varietal characteristic. Normally, cessation of cambial activity proceeds basipetally in the stems of trees (Swarbrick, 1927) and the exception in this case may be correlated with the presence of necrotic areas described below, at the line of union.

The scope of the present investigation only covered two-year-old trees and the fact that abnormalities at the union had not affected tree behaviour is not unexpected in view of the experience of other workers. Armstrong and Brison (1949) describe a graft of *Quercus virginiana* on *Quercus stellata* where breaks began to occur in the union after six years, while symptoms of ill-health only appeared ten years later. Some translocation can probably take place through the parenchymatous areas. Herbert (1927) reports nutritional exchange in natural grafts between trees and lianas in which no vascular connection was established. According to Roach and Thompson (1947) neither vessels nor tracheids appear to be essential for transference of certain dyes across the union. In the incompatible pear/quince combinations studied, there were normal amounts of starch in the root systems of trees indicating that some translocation of sugar had taken place through the discontinuous bark at the union.

The actual amount of vascular continuity necessary for healthy growth of the crown, is very small, as can be observed in wild, partly broken trees. A union may therefore be discontinuous in many parts without affecting the general behaviour of the tree. Symptoms of ill-health due to lack of translocation can only be expected when the amount of connected vascular tissue has fallen below a certain minimum.

It seems likely, however, that structural defects at the union must influence the rate of translocation and the work of Chang (1938) supports this view since he found that incompatible unions increased resistance to the flow of dyes and water.

Although the translocation of food materials across unions with structural defects may be sufficient for satisfactory growth to be made for some years, trees with such unions are subject to the risk of mechanical breakage, the strength of the union depending on the interlocking of the fibres of rootstock and scion.

Microscopical examination of the union.

Abnormal tissues, similar to those described by Proebsting (1926 and 1928) and Bradford and Sitton (1929), were observed in incompatible unions. Radial longitudinal sections were made at the union between rootstock and scion at the base of the graft in one and two-year-old trees. In a compatible union cambial contact was established soon after grafting, and the associated activity of both cambiums produced a continuous vascular connection between rootstock and scion during subsequent growth. A good union in which there was no distortion of the tissues, and where the exact line of union was indistinguishable, is shown in Fig 15*a*. Not all the compatible unions were as even and regular as this one, since slight displacement of the tissues occurred where the cambiums of rootstock and scion were not sufficiently close together at the time of grafting. Where the combination was compatible no distortion due to this cause was visible in the second year's growth.

In sections of the unions of one-year-old incompatible pear trees the vascular cylinder, although sometimes distorted, was not generally interrupted by areas of parenchymatous tissue at the union. There was, however, in some sections a very striking change in the orientation of the xylem tissues at the union. Vessels and fibres instead of following the normal vertical direction were completely horizontal as shown in Fig. 15*b*. Another abnormality observed in some unions and shown in the same photograph is the involution of the wood cylinder and cambial zone at the point of union. It should be noted that the cambium although bent did not appear to be broken at any point. As will be seen in the photograph, vascular connection in this case was only established recently and this may account for the involution.

Definite symptoms of incompatibility were observed in every section of the two-year-old unions of Williams'/Quince B and Clapp's/Quince B. In every one of these discontinuity in the wood started suddenly towards the end of the season as illustrated in Fig. 16. Closer examination showed that the primary cause of the discontinuity appeared to be a necrosis in some cells in the cambial region. The affected zone varied in length. The necrotic cells were adjoining fully differentiated and apparently normal xylem elements. They consisted of both ray cells and fusiform initials not yet fully differentiated (Fig 17*a*). On the phloem side the necrotic cells were in contact with a profusion of large thin-walled callus cells in some of which there appeared to be some meristematic activity, although there was no sign of an organized cambium. Fig. 17*b* shows the end of a medullary ray containing some necrotic cells, while proliferation had taken place from an apparently healthy ray cell. Radial growth had continued above and below the necrotic area, leaving behind a space which was filled by proliferation from adjoining cells. The wood formed by the continuation of radial growth on either side of the necrotic region was abnormal in having no conducting elements. This type of wood extended over a few mm. on either side of the necrotic region.

The bark also showed considerable modifications in structure in both cortex and phloem. A completely new meristematic layer seemed to have arisen in both stock and scion extending through the phloem and part of the inner cortex. A zone of brick-shaped thin-walled cells produced by this meristematic layer, formed a continuous barrier between the phloem of stock and scion. In some cases areas of fully differentiated phloem tissue were found included in this barrier, evidently cut off from once adjacent phloem tissues by the formation of the barrier. The cells of the barrier were not suberized but resembled in many ways the cork cells formed by a phellogen. The orientation and general appearance of this area suggested strongly that the meristematic layer was formed as a sudden reaction of a line of cells after full phloem continuity had been established. Examination of sections of one-year-old unions of the same combination gave only a very slight indication of a similar meristematic area.

Unlike pears, plums showed definite signs of incompatibility in one-year-old unions. Parenchymatous areas between the wood of stock and scion appeared mainly towards the end of the first season's growth, and earlier in some cases. Fig. 18*a* shows a union between Reeves' and Myrobalan B which, at the point of sectioning, had not formed any vascular connection between stock and scion. There was a layer of fully differentiated horizontal tissue at the base of the scion, and a lack of vascular elements in the adjoining part of the stock. Towards the end of the season xylem formation near the point of union ceased, stock and scion becoming separated by an area of non-lignified parenchymatous tissue. Cambial connection between stock and scion had broken down completely, and in this respect many of the plum unions differed from the pear unions in which involution had occurred without, however, affecting the continuity of the fully organized cambium between the stock and scion. Fig. 18*b* represents an extreme case in which there was no vascular connection at the particular point where the section was made. In the section illustrated stock and scion were separated by a cork layer extending through the bark and most of the wood. In this case most of the non-lignified tissue had been formed by the stock, but this is not invariably so, as shown in Fig. 18*c* where the parenchymatous layer was formed equally by stock and scion.

In sections of two-year-old plum unions two different lines of development could be observed. In some cases union was re-established by the formation of a new cambium within the parenchyma layer, rejoining the existing cambiums of stock and scion. Apparently normal radial growth was continued for some time from the re-formed cambium, and an area of unlignified tissues was left behind embedded in the wood (Fig. 18*d*). Cambial continuity was broken again towards the end of the second season with consequent formation of further areas of unlignified tissue. In other cases the tissues of stock and scion continued to be separated by a widening layer of parenchymatous tissue in the second growing season (Fig. 18*c*), although there was generally some evidence of the re-establishment of vascular connection at the beginning of the year. As radial growth continued above and below the point of union, the cambium tended to spread following the outline of the wood, so that eventually there arose two separate horizontal cambiums, belonging respectively to the stock and scion, on either side of the parenchymatous layer. As they continued meristematic activity phloem tissues were cut off causing a widening of the gap between the wood cylinders of stock and scion. The view that the parenchyma layer of older unions consisted at least partly of bark tissues is supported by (1) the presence of crystals typical of bark, and, (2) the seasonal changes in the starch content of this layer which followed those of the bark.

From these descriptions it will be seen that there were certain differences between incompatible unions of the pear/quince and the plum combinations. Discontinuity in the bark of pear unions appeared to be due to a sudden reaction of a layer of cells in the bark, rather than to a disturbance in the cambium. In plums, on the other hand, bark discontinuity seemed to be the result of a general slowing up of growth at the point of union, culminating in a complete disorganization of the cambium of this region and consequent overgrowth by surrounding tissues. While the presence of dead cells next to normal xylem elements in the pear unions suggests a sudden violent reaction which is the beginning of the discontinuity in the wood the more gradual appearance of discontinuity and lack of dead cells in plum unions point to a gradual slowing down of growth, possibly accompanied by modification of the cambium. In plums the break in the continuity of the cambium between stock and scion did not occur equally over the whole circumference of the stem. In some places cambial contact had been maintained, and there were substantial bridges of normally developed xylem tissue across the union (Fig. 13). Tupper-Carey (1930) has shown that the orientation of vascular elements follows the path taken by translocated food substances. If this view is accepted, the direction of the newly-formed vascular tissue above and below the disconnected areas will tend to be towards the places where the union has not been broken, and this may explain the frequent occurrence of horizontally orientated wood tissues in plum unions. In two-year-old pear unions vascular bridges were only rarely observed, and this fact may be associated with the lack of vessels in the wood formed on either side of the union after discontinuity first appeared.

In spite of the differences pointed out above between incompatible pear and plum unions there were also a number of basic similarities suggesting that the primary causes underlying incompatibility in both plums and pears may be similar. In both, the incompatibility was associated with abnormalities in the cambial zone at the union. Disturbances in the cambium seemed to be more pronounced at the end of the growing season, and showed different degrees of intensity at different points in the union.

The suggestion is made that the fundamental causes of incompatibility in the plants examined reside in the neighbouring cells of stock and scion and do not depend on the leaf system. The facts on which this suggestion is based are as follows : (1) in the double-grafted trees the structure of the union between intermediate and rootstock did not appear to be greatly affected by the variety bearing the leaf system and, (2) in incompatible unions only a very limited area of the plant tissues, viz. those immediately adjacent to the point of union were affected in structure.

The evidence available would fit the hypothesis that incompatibility, in the cases investigated, was due to biochemical causes residing within the cell. The seasonal effect observed suggests that the metabolic processes of the tree itself may have some influence on the appearance of symptoms of incompatibility at the union, and that this may be connected with the time at which the maximum downward translocation of elaborated food substances occurs. The suggestion is made that cells of incompatible partners may be able to function together as a unit at certain times of the year but that when they receive some particular elaborated substance, synthesis takes place within the cell of some other substance which creates an antagonism between them, resulting in the breakage of the tissues at the union.

Starch distribution.

The starch content of longitudinal sections of the unions was examined at intervals from December, 1948, onwards. No differences in starch content were seen in December or January, but clear differences in the amount of starch present in the tissues on either side of the union were visible in February. An examination of the starch content of both unions of one tree per combination was therefore planned. The difference in depth of colour appearing when radial longitudinal sections of the unions were treated with iodine was recorded for Trial I (plums) at the end of March and for Trial II (pears) at the beginning of April (Tables XVIII and XIX).

Table XVIII.

Trial I. Plum compatibility.
Starch content of tissues at the union, March, 1949.

Combination.	Top union.		Lower union.	
	Top-scion.	Intermediate.	Intermediate.	Stock.
P/P/MB	1	1	1	4
P/V/MB	2	3	3	4
P/O/MB	1	1	1	4
P/R/MB	2	1	1	4
V/P/MB	2	1	1	5
V/V/MB	3	3	3	5
V/R/MB	2	1	1	4
V/O/MB	3	2	1	4
R/P/MB	1	2	2	4
R/V/MB	1	2	2	3
R/R/MB	1	1	1	3
R/O/MB	1	1	1	4
O/P/MB	1	1	1	3
O/V/MB	1	3	4	5
O/R/MB	2	2	2	4
O/O/MB	1	2	1	4

Category.
1 = low
2 = fairly low
3 = medium
4 = fairly high
5 = high
} starch content.

Table XIX.

Trial II. Pear compatibility.
Starch content of tissues at the union, March, 1949.

Combination.	Top union.		Lower union.	
	Top-scion.	Intermediate.	Intermediate.	Stock.
W/W/QB	3	3	3	4
W/C/QB	4	4	3	4
W/H/QB	3	3	3	3
C/W/QB	4	4	2	4
C/C/QB	4	4	2	4
C/H/QB	3	3	4	4
H/W/QB	4	2	2	4
H/C/QB	4	3	2	3
H/H/QB	3	3	3	3

Category.
1 = low
2 = fairly low
3 = medium
4 = fairly high
5 = high
} starch content.

Where differences in depth of colour occurred the change appeared suddenly at the line of union. Table XVIII shows that large differences in starch content above and below the union were recorded for some combinations, particularly at the lower union between intermediate and rootstock. In all cases the starch content in the rootstock stem was higher than in either intermediate or top-scion. This may be partly the expression of natural gradients existing in all trees, but the differences found cannot be explained on this basis alone, since (1) the total length of stem covered by the sections was only 5 in., (2) the gradient was sometimes interrupted, top-scion and rootstock containing more starch than the intermediate, and (3) the changes occurred abruptly at the line of union. Chang (1938) considered that differences in starch content were due to mechanical obstruction at the union. The present work shows that though both frequently occur together they may not be causally related in the cases examined, for the following reasons, (i) differences in starch content on both sides of the union were not correlated with the degree of structural continuity of the union, (ii) the differences only occurred at certain times of the year and were not apparent during the rest of the year, and (iii) the starch content of the intermediate was sometimes considerably lower than that of either stock or top-scion. Kostoff's explanation of a biochemical specificity is more in accordance with the results obtained, particularly in Trial I (plums) which clearly shows certain varietal trends. When Table XVIII is examined from this point of view variations in starch content of the rootstock and differences between the distal and proximal ends of the intermediate assume particular significance. Such differences occur in the combinations V/O/MB, O/V/MB, O/O/MB among the plums and W/C/QB, C/W/QB, C/C/QB, C/H/QB, H/C/QB among the pears (Table XIX). As judged from the structure of the two-year-old unions all these combinations with the exception of O/V/MB and C/H/QB are likely to be incompatible. In these two there was more starch at the base than at the top of the intermediate whereas the reverse was true for the other combinations. In the pear quince combinations, Table XX, the general level of starch content was higher and the variations were smaller than in the plums. Where the starch content of any part of a tree fell to category 2, it was found that the components of the tree were incompatible. Further evidence of the fact that President and Myrobalan B are not completely compatible is provided by the observations on starch content. The distribution of starch in trees of President on Myrobalan B resembled that of the incompatible combinations.

In October, 1949, a third survey was made of the unions of one tree per combination in Trial I, and two trees per combination in Trial II. All the trees were found to have a high starch content at that time.

Although the evidence of Tables XVIII and XIX cannot be regarded as conclusive since it is based on the results of only one tree per combination, it does indicate that in incompatible trees the starch content of some parts of the tree may be abnormal at certain times of year. The result obtained is of particular interest since these trees had not shown any signs of ill-health up to the time when this record was made. Disappearance of starch from the rootstock of grafted trees showing severe symptoms of ill-health has been recorded in the literature (Fawcett *et al.*, 1946). One such example was investigated in the course of this study. Some four-year-old sweet cherry trees on the rootstock F 250* were dying and it was considered that death was probably due to incompatibility since no other explanation could be found. Fig. 19*a* shows a section of the union of one of these trees (Frogmore/F 250) and Fig. 19*b* shows a similar section treated to indicate differences in starch content. Except for the lack of growth in the stock there appeared to be an entirely uninterrupted vascular connection between stock and scion, and yet there was no starch at all in the stock and a large amount above the union. Trees of Noir de Guben grafted on F.250 showed similar abrupt changes in starch content at the union between rootstock and scion, although the structure of the union was apparently normal. Apart from the sudden constriction at the line of union due to lack of growth in the stock, the union was not obstructed by cork or parenchyma layers in either wood or bark.

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* The rootstock F250 is an acid cherry under trial at East Malling Research Station.

In any consideration of possible correlations between starch content and incompatibility the seasonal variations in the starch content of stems and roots of normal trees must be taken into account. Cameron (1923), Swarbrick (1927), Kraybill *et al.* (1930), and Murneek (1942) record that there are two seasonal minima in the starch content of fruit trees, the first occurring during winter and the second during active growth in summer. The winter minimum is ascribed by Murneek (1942) and others to hydrolysis effected by low temperature. Some time later starch is formed again, and there is a maximum starch content before growth starts in the spring. The records made for Trials I and II appeared to cover the period from minimum winter starch content to maximum content before growth began. It is not at present known what factors control this seasonal starch formation.

PEACH COMPATIBILITY. (TRIAL III.)

GROWTH AND OTHER FIELD OBSERVATIONS.

Trees of Hale's Early peach grafted on two plum rootstocks were included in this study because the symptoms of incompatibility previously observed in young trees were rather different from those usually found in pear or plum. One of the most obvious differences was the sudden cessation of scion growth in the middle of the summer (Garner and Hammond, 1938).

The percentage "take" of grafts was lower in the incompatible combination Hale's on Myrobalan B (55 per cent.) than in the compatible Hale's on Brompton (81 per cent.). Hale's scions made more growth on Brompton than on Myrobalan B rootstocks. Monthly growth records made during 1948, Table XX, showed that Hale's ceased to grow after August when grafted on Myrobalan B, but continued to do so on Brompton.

TABLE XX.

Trial III. Peach compatibility.

Monthly measurement of main shoot of Hale's (cm.).

Combination.	June.	July.	August.	September.	October.
On Myrobalan B (mean of 24 trees)	25	49	69	69	69
On Brompton (mean of 41 trees)	41	73	105	116	119

Table XXI giving the monthly girth increment of the stock stem shows that Myrobalan B increased abnormally little in girth. On the other hand Brompton under the same scion made normal growth. The comparison of girth increment of Myrobalan B stems under compatible plum varieties (Trial I, Table III) and under Hale's is equally striking.

Table XXI.

Trial III. Peach compatibility.

Monthly increment in girth of the rootstock under Hale's (mm.)

Combination.	June.	July.	August.	September.	October.	Total.
Myrobalan B (mean of 24 trees) ..	1	1	1	0	0	3
Brompton (mean of 41 trees) ..	3	3	5	5	1	17

In September, 1948, the leaves of Hale's on Myrobalan B showed a tendency to curl upwards at the margins and developed a reddish colour starting at the edges. The symptoms appeared first on the leaves at the apex of the shoot, and then spread downwards. A defoliation record made on 28th October showed that 80 per cent. of the Hale's leaves had dropped where the Hale's was worked on Myrobalan B, as against 5 per cent. on Brompton.

Defoliation started at the base of the one-year-old shoots in both combinations. The different behaviour of the two combinations at the end of the first growing season, 1948, can be seen in the photograph Fig. 20. During the following winter, 1948-49, a few trees of Hale's on Myrobalan B died, the death of the scion being preceded by that of the stock.

During the second year (1949), Hale's grew very little when grafted on Myrobalan B, but made normal growth on Brompton. In the former combination some of the buds, mainly those situated at the base of the stem, developed shoots up to 12 in. long while die-back of the shoots formed the preceding year was often recorded in the upper part of the plant. The small amount of growth made in the second year after grafting was apparently healthy until August of that year, when the typical curling and red discoloration of the leaves re-appeared, followed by early defoliation.

In May, 1949, the observations on cambial activity, by the Priestley stripping method, showed that the cambium of Hale's on Myrobalan B was less active than that of Hale's on Brompton. The Myrobalan B rootstock under Hale's was completely dormant, while Brompton under the same scion and some ungrafted plants of Myrobalan B showed normal activity of the cambium for the time of year. By the end of June slight cambial activity was detected in the Myrobalan B under Hale's.

HISTOLOGICAL STRUCTURE.

The field records of girth of stem showed that the stem of the Myrobalan B rootstock under the scion Hale's Early peach stopped growing early in the season. The small amount of radial growth made since grafting could be seen clearly in transverse sections of the Myrobalan B stem. Only a narrow annual ring of spring wood with large vessels was formed. Sections taken at the union revealed that the xylem produced immediately after grafting was not normally differentiated. There was a general lack of lignification affecting the fibres in particular.

When the sections were fixed in formalin-acetic acid and stained with Safranine and Fast Green the rays and bark cells of Hale's peach stained differently from those of Myrobalan B and Brompton. The sudden change in colour from purple to green at the line of the union at certain times of year showed that the components of the grafted tree retained to a certain degree their own biochemical constitution.

The samples for the study of the structure of the Myrobalan B wood were chosen from the wood formed in 1947, as the amount of growth in 1948 was so small.

TABLE XXII.

Trial III. Peach compatibility.

Relative area of the elements in the wood.

Type of element.	Combination.	Scion %.	Stock-stem %.	Diff. of means for θ.	
				Scion minus stock-stem.	Sig. diff. (P = 0·05).
Vessels	Hale's/Brompton	14·1	7·9	+5·14	7·65
	Hale's/Myrobalan B	13·4	14·1	−0·63	7·65
Fibres	Hale's/Brompton	68·3	65·8	+1·52	10·75
	Hale's/Myrobalan B	61·1	59·0	+1·27	10·75
Parenchyma cells ..	Hale's/Brompton	0·3	3·5	−8·65	11·14
	Hale's/Myrobalan B	2·9	2·5	+0·88	11·14
Rays	Hale's/Brompton	17·3	22·8	−3·82	7·74
	Hale's/Myrobalan B	22·6	24·2	−1·08	7·74

Size and relative area of vessels, fibres, parenchyma cells and rays in the wood.

No differences occurred between rootstock and scion in the relative area or in the size of any of the four elements in the wood, viz. vessels, fibres, parenchyma cells and rays (Tables XXII and XXIII). One may, however, note that the differences of the means for θ in Table XXII were higher in the compatible combination Hale's on Brompton, than in the incompatible Hale's on Myrobalan B and that for vessels and parenchyma cells they were not far below the value required for significance.

Measurements of rays in the cambial zone.

The scion Hale's and the rootstock Myrobalan B differed significantly in the width of the ray initials in the cambial zone (Table XXIV). Although no difference was observed between the compatible Hale's and Brompton for this character, it is unlikely that it has any direct bearing on the compatibility of the combination, since differences in the width of the ray initials occurred in both compatible and incompatible combinations in the previous trials.

TABLE XXIII.

Trial III. Peach compatibility.

Size of elements in the wood.

Type of element.	Combination.	Scion sq. μ.	Stock sq. μ.	Diff. of means.	
				Scion minus stock.	Sig. diff. (P = 0·05).
Vessels	Hale's/Brompton	1368	1133	+235	549·5
	Hale's/Myrobalan B	1246	777	+469	549·5
Fibres	Hale's/Brompton	119	185	− 66	126·9
	Hale's/Myrobalan B	105	98	+ 7	126·9
Parenchyma cells ..	Hale's/Brompton	163	170	− 7	298·6
	Hale's/Myrobalan B	132	177	− 45	298·6

TABLE XXIV.

Trial III. Peach compatibility.

Distance between rays, and width of ray initial for uniseriate rays.

	Combination.	Scion μ.	Stock μ.	Diff. of means.	
				Scion minus stock.	Sig. diff. (P = 0·05).
Distance between rays	Hale's/Brompton	46·0	53·1	+ 7·1	24·5
	Hale's/Myrobalan B	31·9	52·4	−20·5	24·5
Width of ray initial in uniseriate rays	Hale's/Brompton	14·8	13·3	+ 1·5	3·8
	Hale's/Myrobalan B	13·4	9·3	+ 4·1	3·8

Phloem structure.

When an examination of the phloem structure was made in October, 1948, the phloem of both scion and rootstock of trees of Hale's on Brompton appeared to be normal, but certain abnormalities in the phloem were observed in trees of Hale's on Myrobalan B. The phloem of the Myrobalan B stem just below the union showed a necrotic condition similar to that described by Schneider (1945) in peaches with buckskin virus disease. Sieve tubes and companion cells were mainly affected. They were completely collapsed in the older phloem, leaving areas which stained deeply with Fast Green and which contained the remains of cell walls and contents. Some small cells with entire walls, probably parenchyma, remained behind as islands in the disorganized tissue. The more recently formed phloem nearest to the cambium was also abnormal and did not seem to be fully differentiated. The ray cells and the cambium itself appeared to remain normal. The total amount of phloem produced seemed rather large but the cells themselves were abnormally small. In order to study the distribution of the affected phloem, other sections were cut of the Hale's stem at the middle of the shoot and just above the union, of the Myrobalan B stem just below the union and below ground level, and of the roots. In all sections phloem development was abnormal, though the necrosis did not appear to be equally advanced in all. It was most severe in the sections of the Myrobalan B rootstock, less so in the Hale's just above the union, and least half-way up the stem.

Considering the disorganized state of the phloem over most of the tree and particularly in the rootstock it was difficult to understand how the Hale's on Myrobalan B could continue to live for another year. When, however, it became apparent, that these trees were growing, it was decided to observe further developments in the phloem during the second season. Sections cut at monthly intervals from July to October, 1949 in five positions on one tree per month, gave an indication that a partial recovery had occurred at the beginning of the second growing season in both stock and scion, to be followed by an evident necrosis towards the end. Although the boundary between the collapsed phloem of the previous season and the necrotic areas in the current season's phloem was not clear, it seemed that some necrosis of the phloem occurred before symptoms of ill-health appeared in the leaves. A regrafting experiment described below showed that the cambium in affected trees is capable of forming apparently healthy phloem again when the incompatible scion is removed.

There appears to have been little previous work on the structure of the phloem in connection with incompatibility studies with plum rootstocks, but symptoms of phloem necrosis have been described by Schneider (1947) and Oberholzer (1947) for incompatible combinations in citrus. In order to discover whether the same kind of necrosis occurred in incompatible plum/plum combinations as in the peach/plum combinations, a section of a Myrobalan B root from a six-year-old tree of Victoria/President/Myrobalan B exhibiting symptoms of ill-health was examined, and it was found that similar phloem abnormalities were indeed present. The total amount of phloem was larger than in a similar root from a compatible Victoria/Victoria/Myrobalan B tree. Premature necrosis of the phloem was evident.

These observations on phloem abnormality have indicated the possible value of future research on the development and structure of the phloem in the roots of incompatible combinations to discover the exact sequence of the three conditions found to be associated with incompatibility, viz. phloem necrosis, starch differences and symptoms of ill-health visible in the field.

INTERNAL EXAMINATION OF THE UNIONS.

Macro- and microscopical examinations of the unions were carried out in the same way as for the previous trials. The unions between the incompatible varieties Hale's and Myrobalan B, differed notably from those of the compatible Hale's and Brompton. The overgrowth of the scion at the union was evident at first sight in the Hale's on Myrobalan B trees, and in some places where the edges of the stock and scion were not matched the ingrowing bark of the scion was pushing the components apart. Nevertheless good vascular connections between stock and scion were established in both bark and wood over much of the union and this fact made the unions mechanically strong. Discontinuity either in wood or bark, when present, seemed to be a result of the ingrowing bark and consequent separation of stock and scion.

It is probable that the defects at the union between Hale's and Myrobalan B were not primarily responsible for the symptoms of ill-health in the scions. The amount of abnormal tissue at the union was no greater than in the plum and pear trials where no external symptoms of ill-health were observed and, in addition, the degree of abnormality at the union was variable, while the time of occurrence and the character of the symptoms of ill-health were constant.

Starch distribution.

In the trees examined in September, 1948, the distribution of starch presented striking differences. In the trees of Hale's on Brompton both scion and rootstock contained much starch, the amount being lower in the scion ; while in the trees of Hale's on Myrobalan B the starch had disappeared from the roots, was present to a small degree in the Myrobalan B stem below the union, and in much greater amount in the scion. The lack of starch in the roots of the incompatible Hale's Myrobalan B was checked in nineteen trees during October with the same result. The roots of ten trees of Hale's on Brompton and some ungrafted Myrobalan B plants contained much starch at that time. The scion Hale's had a higher starch content when grafted on Myrobalan B than when grafted on Brompton. During the following February, the same differences in starch content were recorded in the rootstocks Myrobalan B and Brompton under Hale's, while there were no obvious differences between the scions in the two combinations.

During the second year's growth (1949) monthly observations showed the presence of very small amounts of starch in the roots of Myrobalan B under Hale's in August, September and October. No starch was found in the roots in November.

In order to study in more detail the disappearance of starch from the roots of Myrobalan B under Hale's during the first growing season after grafting a small trial was designed as follows : twelve Myrobalan B rootstocks were grafted in the spring of 1949 with Hale's scions. Four grafts failed to " take " and the rootstocks were allowed to grow. Half the remaining trees were allowed to produce Myrobalan B sucker shoots, while the other half bore only the Hale's foliage. Myrobalan roots were therefore growing under (1) Myrobalan B foliage, (2) Hale's foliage, (3) Myrobalan B and Hale's foliage. One small root of each tree was excavated at monthly intervals during the growing season, and sections from it tested for starch. At the time of active growth in June, starch disappeared from the roots of all three types of tree. In August and September starch was again found. In the roots of trees with all or part of the foliage provided by Myrobalan B the amount of starch was high but it was low in two of the four trees with Hale's foliage only, and absent in the other two. The typical symptoms of ill-health described above developed on the Hale's foliage in September whether or not starch was present in the roots. The trees were lifted in November and the survey of starch revealed that (1) the ungrafted Myrobalan B had much starch in the root system, (2) the Myrobalan B under Hale's foliage had no starch in the roots, but some in the stem below the union, (3) the Myrobalan B under Hale's with sucker shoots of Myrobalan B, had much starch in the roots and less in the parts above the Myrobalan B sucker shoots.

The absence of starch in the roots of Myrobalan B under Hale's, after the summer peak of scion growth, suggests that there may have been a lack of translocation to the roots of the materials synthesized in the aerial parts of the tree.

McClintock (1948) studying incompatibility between peaches and Marianna plum found that the roots died first and that the phloem tissues of the plum stock and the peach scion failed to unite, and he concluded that this mechanical obstruction at the line of union caused a lack of translocation to the roots leading to their death. In the present case, it is unlikely that this particular type of mechanical obstruction at the line of the union was the primary cause of the disappearance of the starch from the roots since, (1) the union of Hale's Myrobalan B presented in general better connections than some of the incompatible unions in Trials I and II where a lack of starch in the roots was not observed, and, (2) there was a variation in the quality of the unions which was not correlated with the disappearance of starch in the Myrobalan B rootstock under Hale's.

On the other hand, the disappearance of starch in the root system seemed to be associated with the period when the greatest amount of necrosis appeared in the phloem tissues at the end of the summer. A one-year-old tree of Hale's Myrobalan B, in which the rootstock was allowed to develop sucker shoots, was examined for phloem necrosis. The parts of the rootstock below the sucker shoot showed slight indications of early collapse of the older phloem, but there were no necrotic sieve tubes in the younger phloem. In the piece of Myrobalan B stem between the union and the sucker shoots, early collapse of the older phloem was more conspicuous. The amount of starch was higher in the parts below the sucker shoots and this seems again to suggest a correlation between disappearance of starch and premature phloem necrosis.

The type of incompatibility between Hale's and Myrobalan B bears a striking parallelism with the quick decline disease in citrus. A short historical review of the disease has been given in the introduction. The main features are, (1) the necrosis of sieve tubes first in the stock below the union and then spreading above and below, and (2) the disappearance of starch from the root system in the early stages of the disease (Fawcett *et al.* 1946). Sobrinho (1946) and McAlpin *et al.* (1948) put forward the hypothesis that the necrosis in the phloem produces the lack of starch in the roots, and the subsequent starvation of the roots produces the symptoms of ill-health in the leaves. In the Hale's/Myrobalan B trees the necrosis in the phloem tissue could be responsible for the disappearance of the starch since the two seem to be closely correlated. On the other hand, starvation of the roots does not seem to be entirely responsible for the appearance of the symptoms of ill-health in the top, since trees with rootstock suckers, where the root systems were well provided with starch, developed symptoms of ill-health in the Hale's foliage at the same time and with the same intensity as those without rootstock suckers and with no starch in the root system. It must be remembered, however, that in these trees the piece of rootstock stem between the roots and the union showed necrosis of the phloem and contained less than the normal amount of starch.

Pearsall (1949) points out that a high concentration of sugar may result in the development of anthocyanins. In the Hale's on Myrobalan B trees there was an accumulation of food substances that was not translocated to the roots and it is possible that this accumulation was responsible for the appearance of red coloration in the leaves.

A spectroscopic analysis, made in September, 1948, showed that a larger amount of potassium and smaller amounts of calcium, iron, magnesium and manganese were present in abnormal Hale's leaves from incompatible trees on Myrobalan B than in normal leaves of Hale's growing on Brompton.* No explanation can be offered for this fact but it is interesting to note that the amount of potassium has been found to be correlated, in some plants, with the condensation of reducing sugars to starch (Sideris, 1945) and with the formation of the abscission layer resulting in early defoliation (Chandler, 1936). In the incompatible trees in the present experiment early defoliation of the scion and accumulation of starch in the scion tissues were associated with an unusually large amount of potassium.

POSSIBILITY OF A VIROLOGICAL EXPLANATION OF INCOMPATIBILITY.

The presence of latent viruses or virus complexes in stone fruits has been demonstrated by Milbrath *et al.* (1945) and cases of viruses being responsible for the failure of a particular scion/stock combination have been mentioned in the introduction. Since the type of incompatibility in the Hale's/Myrobalan B combination bore some resemblance to that in plants where a virus has been shown to be responsible for the abnormality, a preliminary investigation on the transmissibility of symptoms was carried out in the following way: scions of Hale's grown on Myrobalan B during 1948 were grafted in the spring of 1949 on Hale's growing on Brompton. The four top-grafted trees made normal growth during 1949 and did not show any symptoms of ill-health at the end of the summer. During the same spring (1949) the Hale's scions were removed from four one-year-old Hale's/Myrobalan B trees and the Myrobalan B stocks regrafted with scions of Myrobalan B. In two of these regrafted trees the rootstock died before union took place, while the other two made good growth during 1949. No symptoms of ill-health were revealed in the scions. Starch tests and microscopical examination of the phloem of the root systems showed that normal phloem was formed after the Hale's scions were removed.

This result permits the conclusion that if there is a virus involved in the incompatibility of Hale's and Myrobalan B, it shows only when both varieties are grafted together. The case would be similar in some respects to those described in the introduction where the sour orange rootstock for citrus, Mahaleb for cherries, and Spy 227 for apples were involved.

DISCUSSION.

The observations made on the nursery behaviour of the incompatible combinations described above suggest that they may be regarded as being in at least two different groups:

(1) Combinations showing symptoms of incompatibility only at the graft unions, the general behaviour of the young trees not being visibly affected.

(2) Combinations showing, in addition to symptoms at the union (i) decrease in growth, (ii) external symptoms of ill health in the leaves and early defoliation, (iii) premature degeneration of the phloem, (iv) abnormal distribution of starch. It is considered that in all probability abnormalities at the line of union are not directly responsible for the other disorders.

The first type of incompatibility was observed in the plum and pear/quince combinations studied in Trial I and II, and the second type occurred in the peach/plum trees studied in Trial III.

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* The writer is indebted to the Biochemical Section at East Malling Research Station for this analysis.

PLATE I.

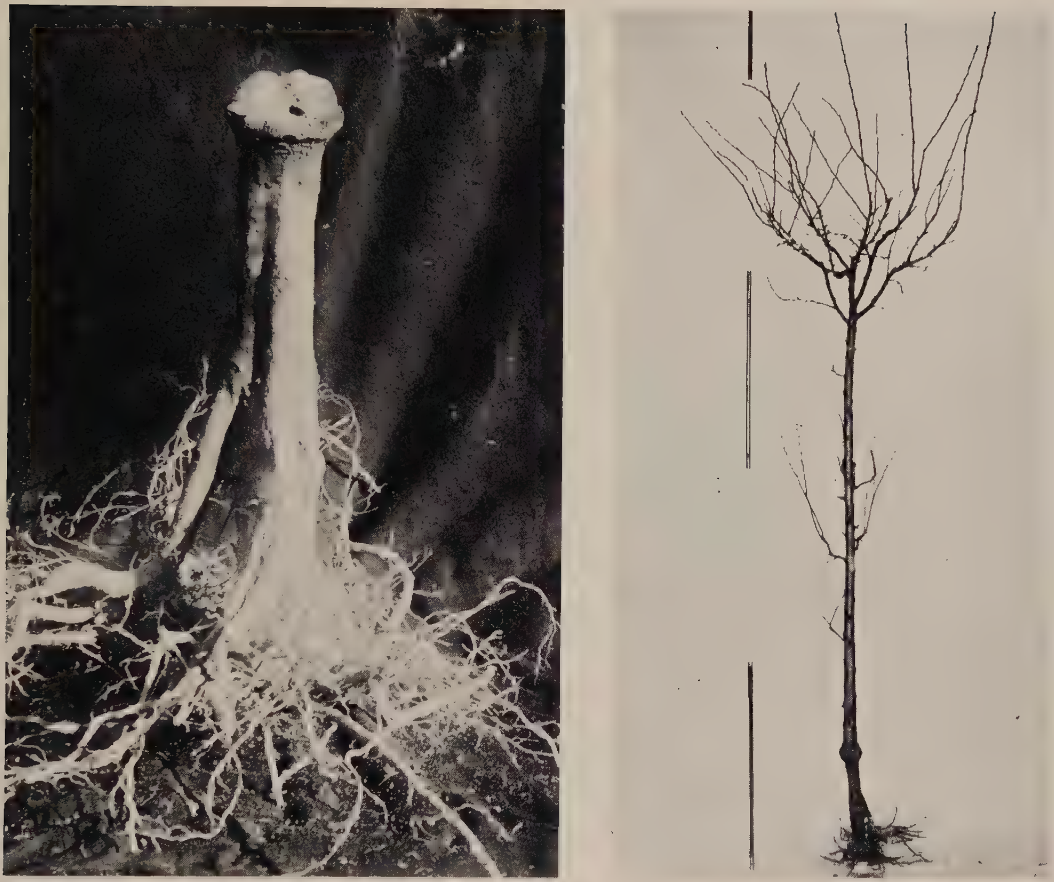


Fig. 1.

Four-year-old tree of Reeves' Seedling on Myrobalan B. On right, tree after lifting; on left, rootstock broken off at the graft union. The clean smooth type of break is characteristic of incompatible unions.

PLATE II.

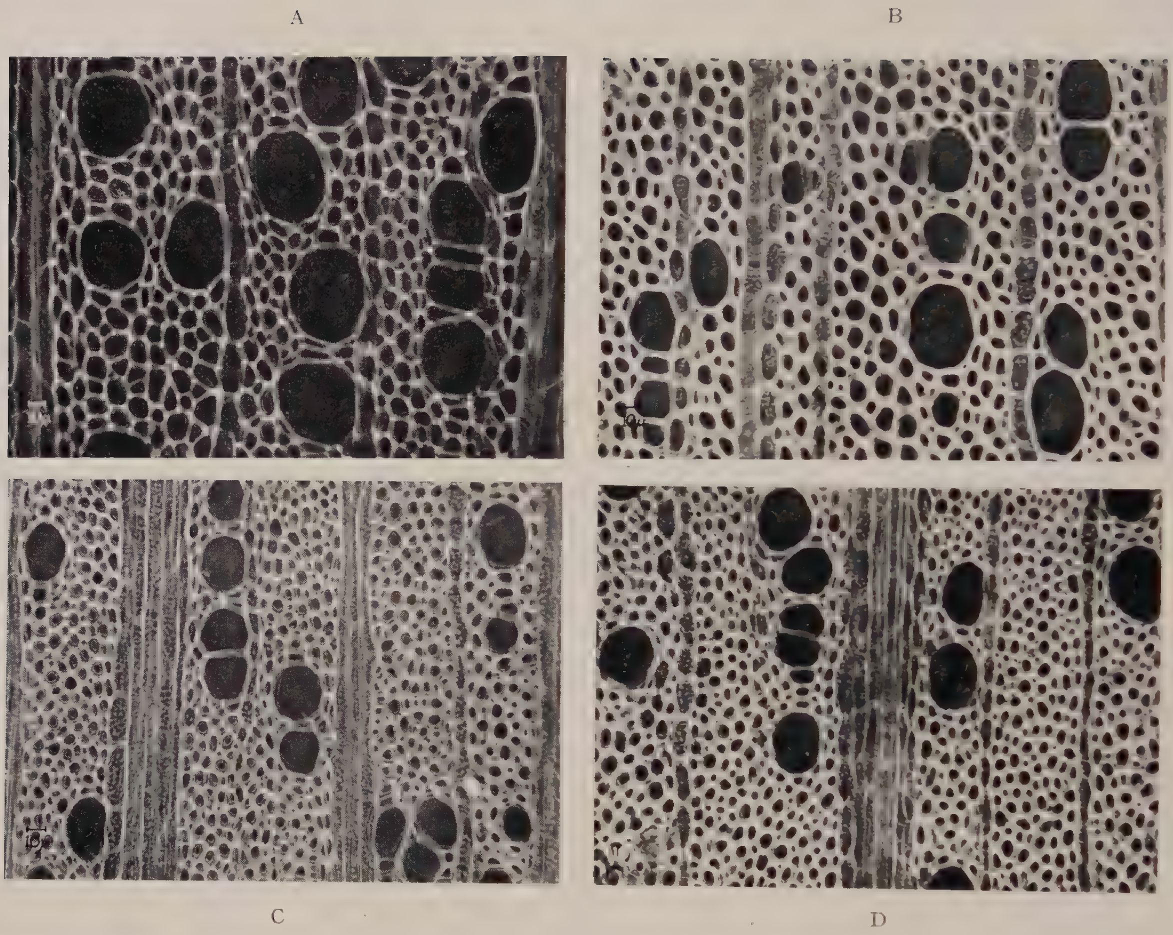


FIG. 11.

A Victoria (on Myrobalan B)
C Myrobalan B (under the scion Victoria)

B Oullins (on Myrobalan B)
D Myrobalan B (under the scion Oullins)

Photomicrographs of transverse sections of the wood of stems Differences in vessel area and size are larger between the compatible varieties Victoria and Myrobalan B than between the incompatible plants Oullins and Myrobalan B

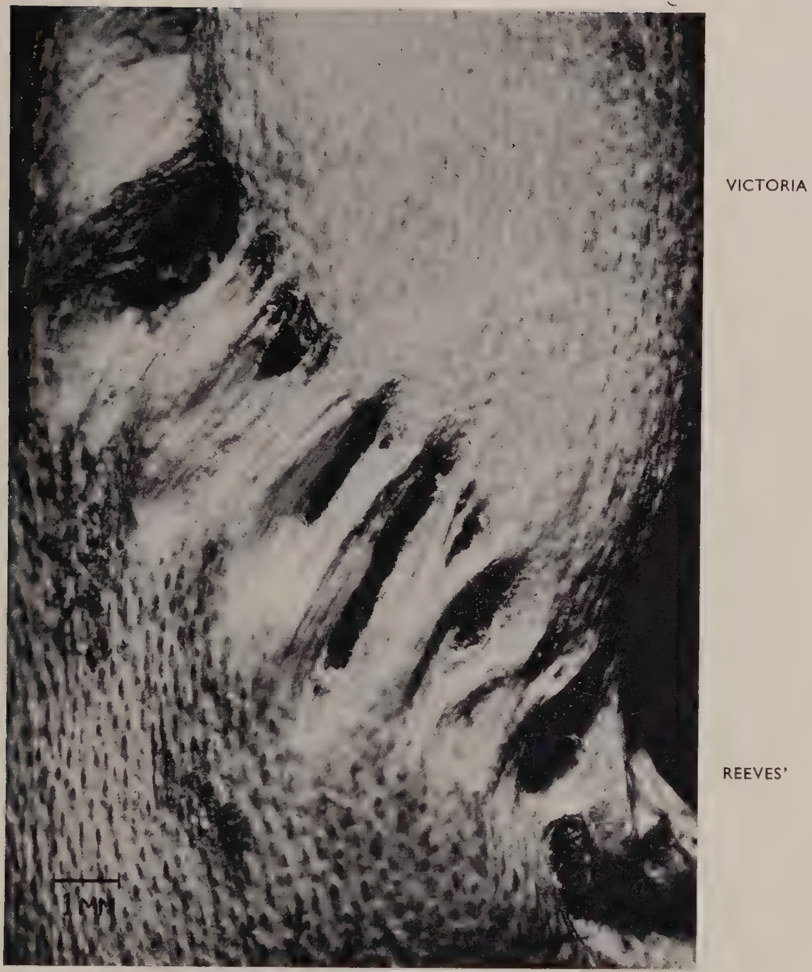


FIG. 13.

Photomicrograph of a union between Victoria and Reeves' Seedling in a double-grafted plum tree on Myrobalan B rootstock. The photomicrograph, taken after the removal of the bark, shows the bridges of wood between the spaces that were once filled with non-lignified parenchymatous tissue. The affected areas are mainly located on the Reeves' intermediate.

PLATE IV.

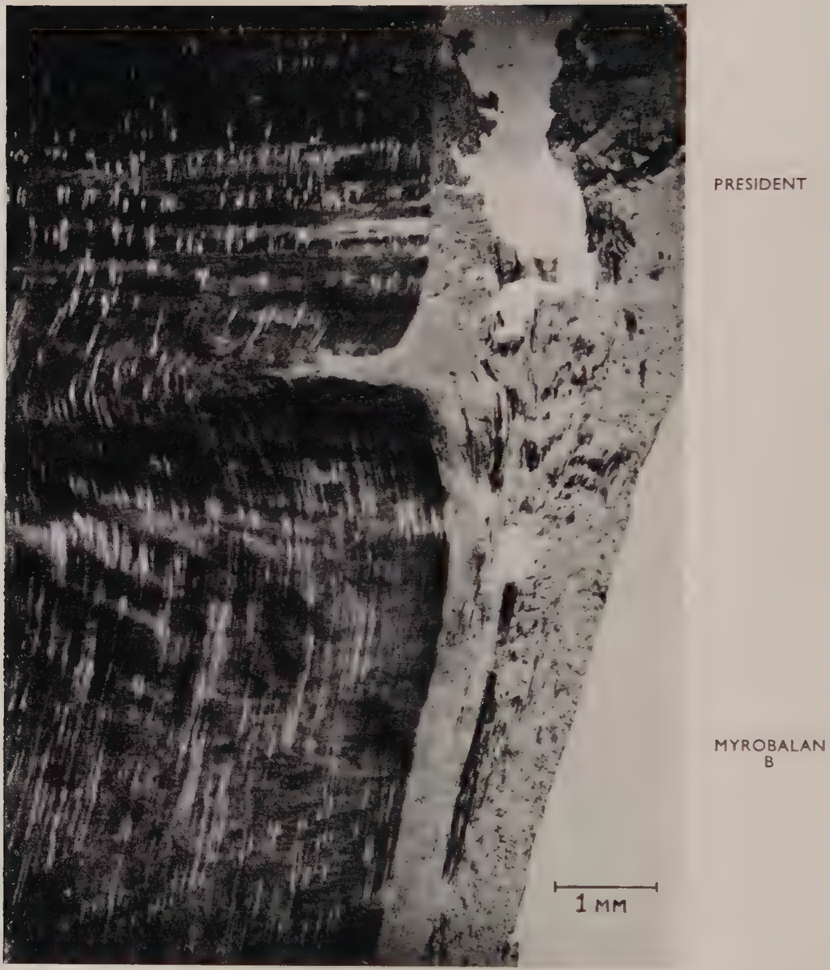
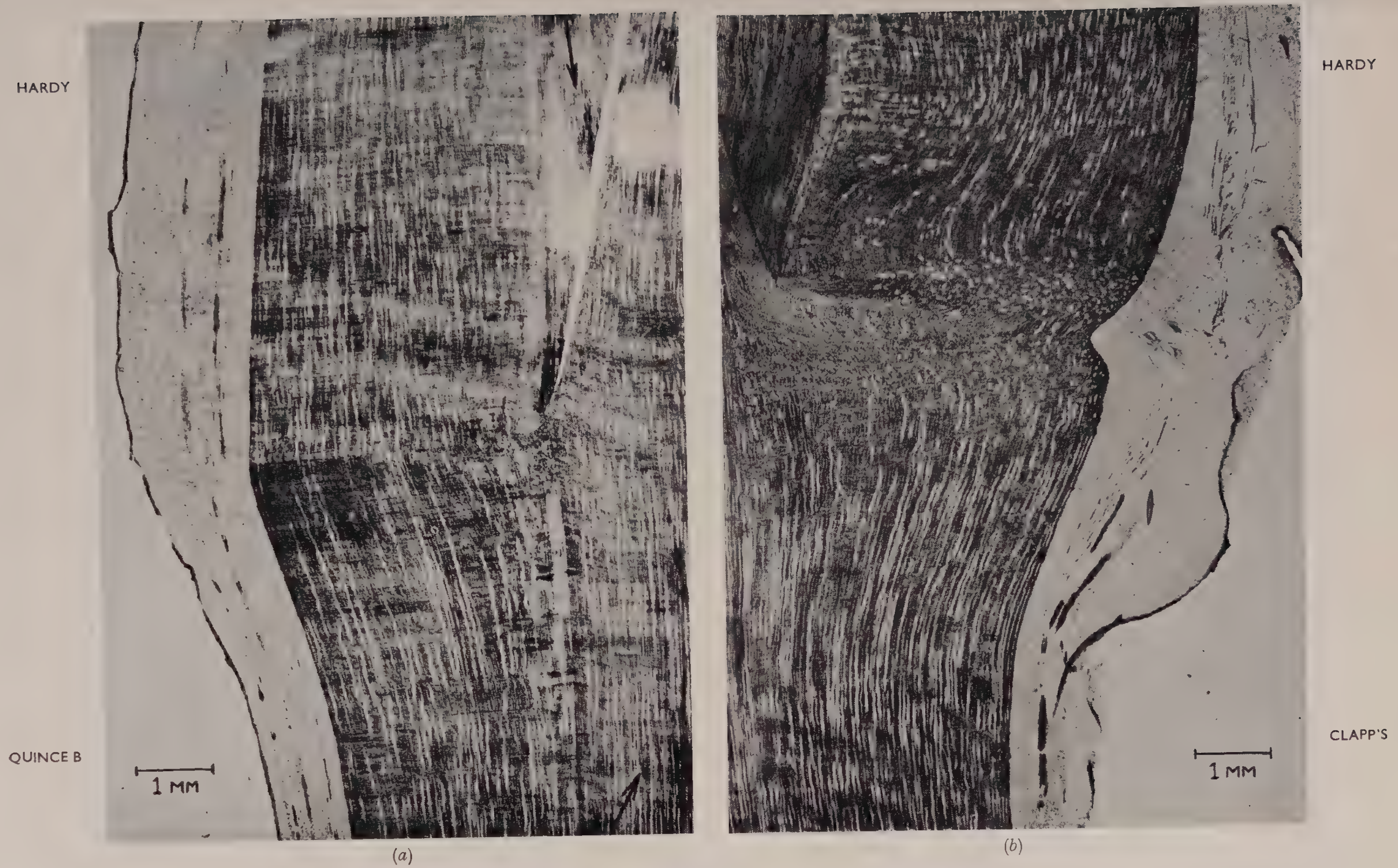


FIG. 14.

Radial longitudinal section of a union between President and Myrobalan B in a double-grafted tree of the combination P/P/MB. The slight discontinuity in the wood at the end of the second year, indicates some degree of incompatibility.



(*a*) (*b*)

FIG. 15.

(*a*) Radial longitudinal section of a one-year-old union of Hardy and Quince B in a tree of the combination W/H/QB. The union of the cambium of stock and scion was established soon after grafting and the associated activity of both cambiums has produced a perfect vascular connection during subsequent radial growth.

(*b*) Radial longitudinal section of a one-year-old union of Hardy and Clapp's in a tree of the combination H/C/QB. Notice the horizontal tissue formed after grafting and involution in the cambial zone at the end of the season. Unions of the same combination did not develop definite symptoms of incompatibility during the second year's growth.

PLATE VI.

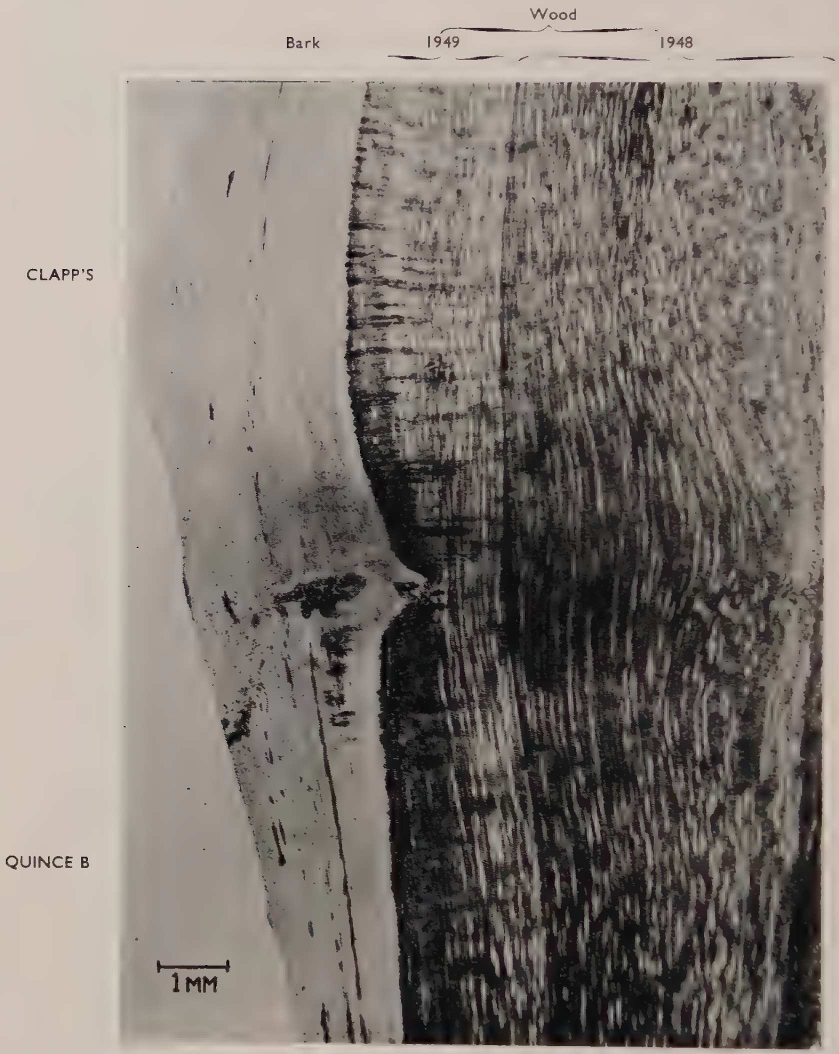

Wood
Bark
1949
1948
CLAPP'S
QUINCE B
1 MM

Fig. 10.

Radial longitudinal section of a two-year-old union of Clapp's and Quince B in a tree of the combination H/C/QB. Vascular continuity exists between rootstock and scion in the wood formed during 1948 and part of 1949, but the continuity is suddenly broken at the end of the 1949 growing season

PLATE VII.

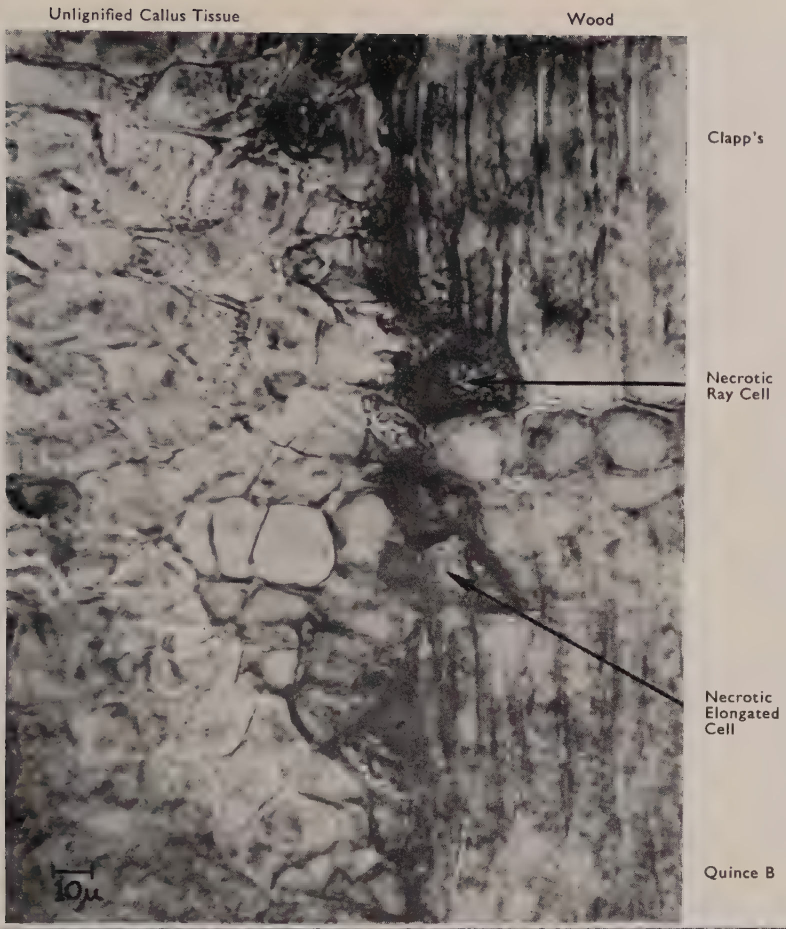

Unlignified Callus Tissue
Wood
Clapp's
Necrotic Ray Cell
Necrotic Elongated Cell
Quince B
10μ

FIG. 17*a*.

Photomicrograph of radial longitudinal section at the union between Clapp's and Quince B showing necrotic cells in contact with fully differentiated wood elements. On the left-hand side are thin-walled callus cells some of which show meristematic activity.

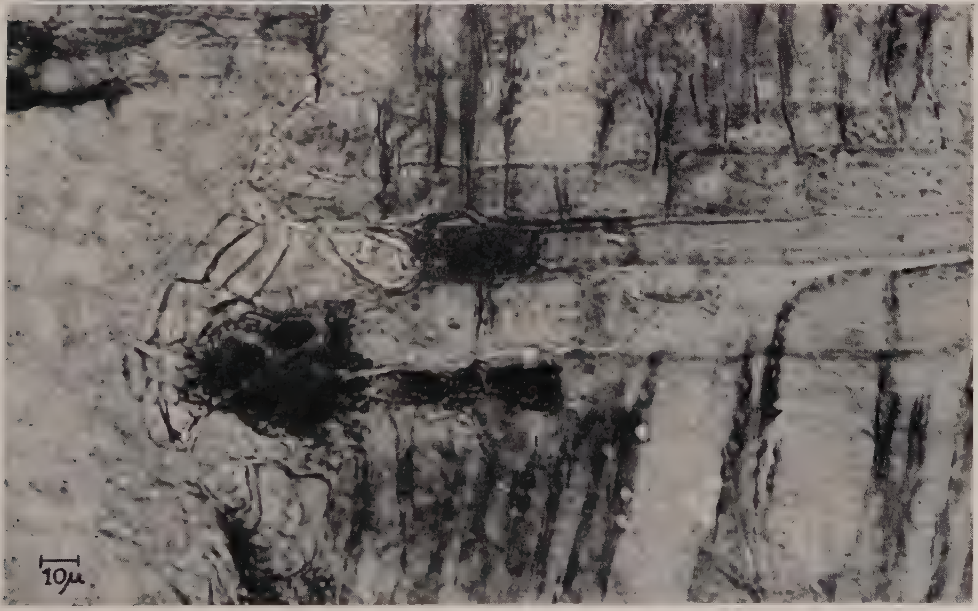


FIG. 17*b*.

Photomicrograph of the zone of union between Clapp's and Quince B where the cambium was destroyed. A medullary ray containing necrotic cells and some proliferating cells is visible. In the necrotic cells the presence of circular bodies can be observed.

PLATE VIII.

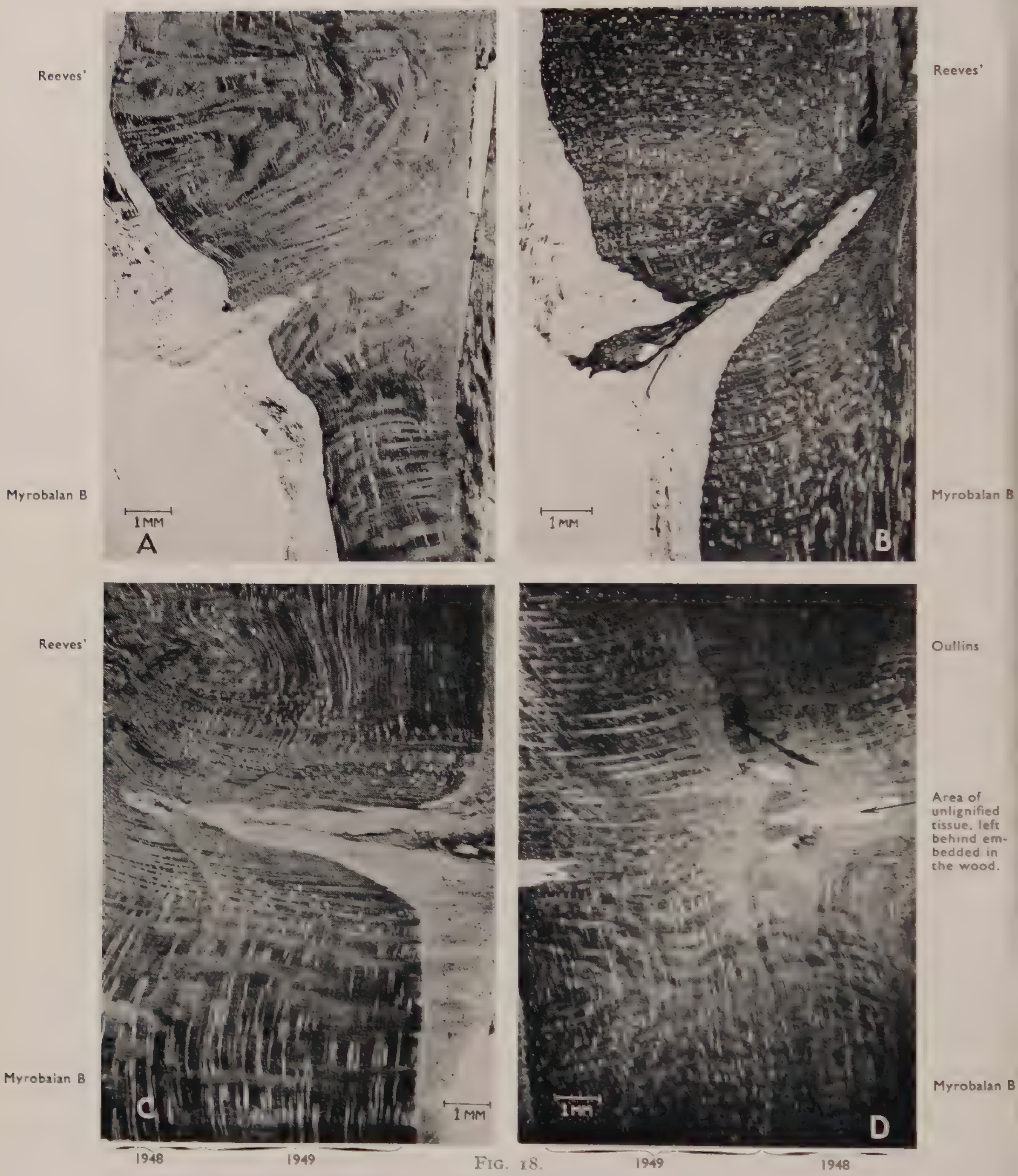

Reeves'
Myrobalan B
1 MM
A
Reeves'
Myrobalan B
1 MM
B
Reeves'
Myrobalan B
C
1 MM
1948
1949
Oullins
Area of unlignified tissue, left behind embedded in the wood.
Myrobalan B
1 MM
D
1949
1948

FIG. 18.

(A) Radial longitudinal section of a one-year-old union of Reeves' and Myrobalan B in a tree of the combination V/R/MB.

(C) Radial longitudinal section of a two-year-old union of Reeves' and Myrobalan B in a tree of the combination P/R/MB.

(B) Radial longitudinal section of a one-year-old union of Reeves' and Myrobalan B in a tree of the combination O/R/MB.

(D) Radial longitudinal section of a two-year-old union of Oullins and Myrobalan B in a tree of the combination P/O/MB. The union was re-established at the beginning of 1949 and broken again some time later.

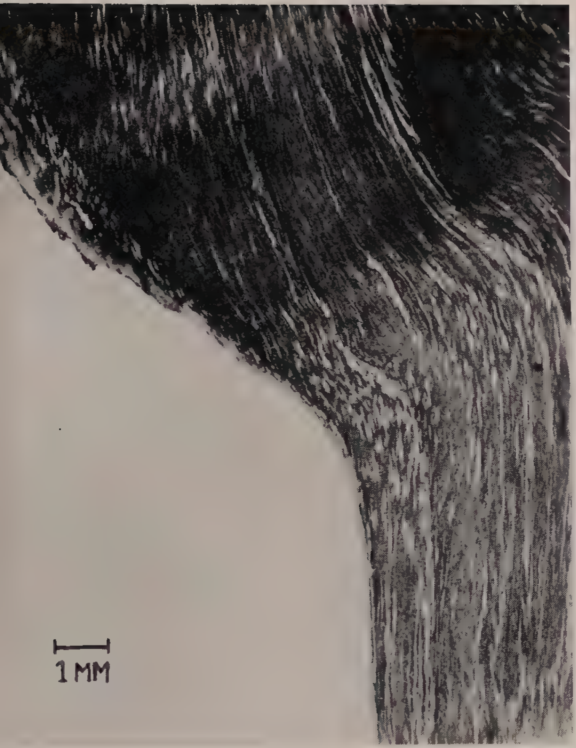


FIG. 19*a*.
Radial longitudinal section of the graft union of a cherry tree Frogmore on F250.

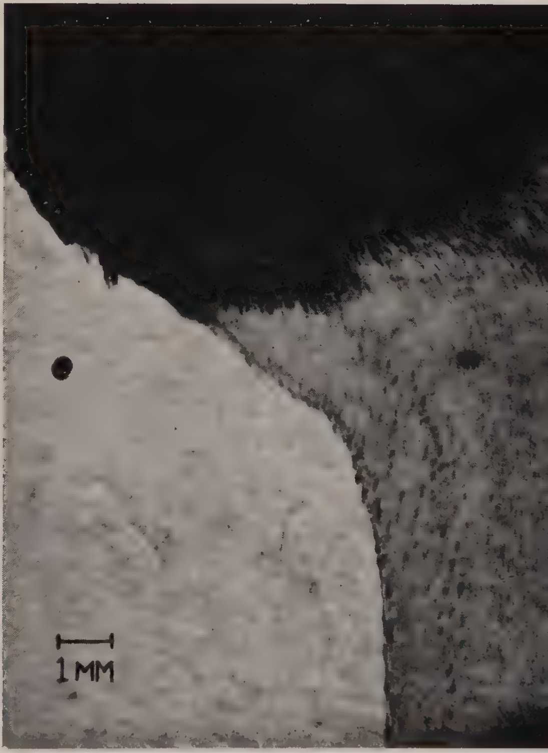


FIG. 19*b*.
A section of the same union as in (*a*) treated with iodine to show starch content.

PLATE X.

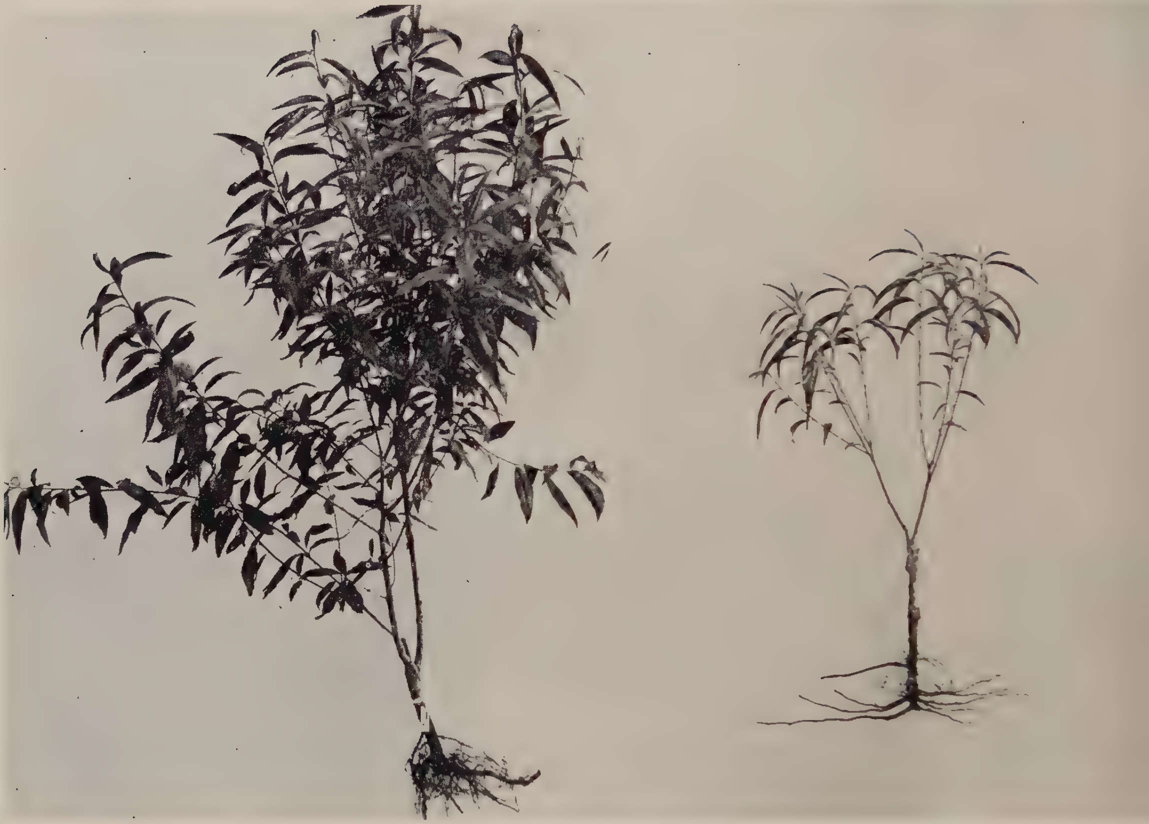


FIG. 20.

Hale's Early peach grafted on Brompton (left) and on Myrobalan B (right). The first combination is compatible and the second incompatible. The trees were grafted in March, 1948, and the photograph taken the following September. The tree on the right shows restricted growth and early defoliation.

Whether the second type of incompatibility is an accelerated form of the first type is a question that can only be answered by observations on the first type over a long period of years. In the light of present knowledge it seems as if the two types were completely different and due to two different causes. Experience has shown that some combinations, known to develop an abnormal structure at the union soon after grafting, do not develop external symptoms of ill-health for a considerable period of years. Oullins on Myrobalan B among the plums and Williams' on quince among the pears are examples of this type of tree. The failure of trees of these combinations is usually due to breaking off at the union during gales.

When an incompatible tree showing structural defects at the union also shows symptoms of ill-health, there is a tendency to consider the first disorder as a primary cause of the second. And certainly girdling effects due to incompatible unions are conceivable explanations for decay of trees. On the other hand when in incompatible combinations, such as Hale's on Myrobalan B, the appearance of considerable abnormalities in growth and distribution of food reserves is not in proportion to the possible girdling effect of the graft union, one must conclude that an additional cause is mainly responsible for such disorders. In some citrus plants similar disorders have been shown to be due to a virus and such an explanation is not ruled out in the case of Hale's on Myrobalan B, where the effect on the metabolism of the whole plant is not unlike that in citrus plants where the failure is ascribed to virus infection. Infectivity tests are therefore important as one of the first tests in future investigations. Crane (1945) considers that viruses can be originated in grafting " by the invasion of the cells of one variety by the proteins of another ". " What is a stable and presumably useful cell protein with one plant genotype acts as a destructive agent with another ", writes Darlington (1944) on the same subject. In the case of Hale's on Myrobalan B the rootstock was more seriously affected in rate of growth, starch content and phloem development than the scion and this would fit the hypothesis that the stock was first affected, the damage in the scion being an after effect. Since, as soon as the scion was removed, the stock rapidly came back to normality, one could suppose that the scion was introducing into the stock some poisonous principle responsible for its decay. Whether this supposed poisonous factor was a virus, or was developed by incompatibility, or was an ordinary metabolic product of the scion, such as the normal proteins, is not known.

The observations on vegetative growth did not point to any of the features studied as being directly associated with the basic causes of incompatibility, although growth can be seriously affected by incompatibility, as seen in the reduction of shoot elongation of the scion and of the radial growth of the stock in the combination Hale's on Myrobalan B.

The cambial meristem seemed to be involved in the manifestation of symptoms of incompatibility. When the union was discontinuous, the alteration and even destruction of the cambium at the zone of contact of stock and scion seemed to be mainly responsible for the structural disturbances observed. Differences in periods of cambial activity in stock and scion, however, do not seem to be among the primary causes of incompatibility, since in spring the cambial activity, in trees where the stems were formed by compatible and incompatible varieties, developed in a normal basipetal way, and this was also true where the rootstock was known to be earlier than the scion when ungrafted. In the combination Hale's on Myrobalan B the rootstock was much later than the scion in starting cambial activity in spring, but this could not be explained on the basis of varietal difference, in this character, since the ungrafted Myrobalan B had an active cambium, at the same time showing that the retardation of cambial activity in the Myrobalan B under Hale's may have been an effect of the incompatibility.

One of the aims of the present studies was to find out whether any correlation existed between histological characteristics and compatibility. If there were such a correlation it would be useful in forecasting incompatibility. The comparison of the structure of compatible and incompatible varieties did not, however, reveal any associations between structure and compatibility. Some nine histological characteristics studied in the wood and in the cambial zone, gave negative results in this respect. Although other histological characteristics may possibly be correlated with incompatibility, the negative results obtained on the nine studied probably indicate that the basic causes of incompatibility are not due to differences in the structure of the varieties grafted.

The lack of association between incompatibility and any of the above-mentioned features as well as the differences found in the starch concentration and the microscopical observations on incompatible unions, point to the biochemical field as a promising line for future research. In this connection it is interesting to note that the components of the graft unions appear to maintain to a certain degree their biochemical individuality, as revealed by the starch tests and by differences in oxidation, and of the staining of the tissues in contact. Many substances in cells can be detected by micro-chemical tests (Johansen, 1940) and the application of such tests to sections of compatible and incompatible unions, made at various periods of the year, could be of help in future investigations on the cause of incompatibility.

The present investigation has shown that symptoms of incompatibility tend to appear, and to reappear, in the unions towards the end of each growing season and that they do not necessarily appear simultaneously and with equal intensity in all the trees of a combination, nor even at all points within a particular union. Both the greater degree of incompatiblity at the end of the season and the varying intensity of the reaction at different points in the union could be explained on the supposition that some internal reaction occurs within or near the cambial cells at the union due to certain elaborated substances moving downwards towards the end of the growing season. Variation in the amounts of these elaborated substances, coming from different branches, could explain the change in the intensity of the reaction at different points of the union. The study of trees where the top scion and intermediate have different degrees of compatibility with the rootstock has indicated that, if this is correct, these elaborated substances may be supplied by the leaf system of both compatible and incompatible varieties and the way in which the cells at the union transform them is the important factor in the development of the latent antagonism giving rise to discontinuity at the line of union.

SUMMARY.

1. The problem of incompatibility between rootstock and scion in a range of plants is discussed. A review is given of such of the literature on incompatibility as appeared to be relevant to the present study of this problem in fruit plants.

2. The behaviour of compatible and incompatible graft combinations is described for three randomized field trials as follows :

> *Trials I and II.* Four plum and three pear varieties known to differ in their degree of compatibility with the rootstocks used, double-grafted in the spring of 1948 (Trial I plums), (Trial II pears).
>
> *Trial III.* Single-grafted trees of one peach variety on two rootstocks possessing different degrees of compatibility with the scion.

3. Studies on (i) time of bud-break, (ii) initiation and basipetal spreading of cambial activity in spring, (iii) amount and time of shoot and trunk growth, did not suggest that these characteristics were directly associated with the primary causes of incompatibility between rootstock and scion, although in one of the trials studied these features were seriously affected by incompatibility.

4. A sample of the trees from the field trials was used for a study of the internal structure of the different components of the double- and single-grafted plants.

Nine histological characteristics were studied in tranverse sections of different parts of the stems and roots, i.e. the relative amount of vessels, fibres, parenchyma cells and rays in the wood, the size of the first three of these elements, and the width of ray initials and distance between rays in the cambial zone.

Differences in histological structure between the components of the grafted trees were found in many combinations, but such differences were not correlated with the degree of compatibility of the varieties grafted. No effect of the scions was apparent on the structure of the rootstocks.

5. The same sample of trees referred to in the paragraph above and, in addition, further samples, were used for macro- and microscopical observations of both compatible and incompatible unions.

The upsetting and even the destruction of the cambial meristem at the union between stock and scion appeared to be responsible for a layer of undifferentiated tissue occurring between the stock and scion. In general this abnormality tended to appear at the end of the growing season and to occur with varying degrees of intensity at different points in the union. The probable effect of the undifferentiated layer of tissue at the union on the mechanical strength of incompatible combinations and on the translocation of food materials is discussed.

The manifestation of symptoms of incompatibility in the plum and in the pear/quince combinations differed. In the former there was considerable variability within each union and also between different trees of the same combination, while in the latter the amount of variability was much less.

6. Differences in starch distribution between different parts of grafted trees suggested that the study of the starch metabolism may be important in connection with incompatibility between rootstock and scion.

7. Two different forms of incompatibility were observed: (1) where abnormalities occurred mainly at the graft union and, (2) where the following symptoms occurred in addition to those at the union, (i) restricted vegetative growth, (ii) signs of ill-health in the leaves accompanied by early defoliation, (iii) premature degeneration of the phloem, (iv) abnormal distribution of starch.

The first type was observed in the plum and in the pear/quince combinations and the second in the peach/plum combinations. The possibility that these two forms may be due to different primary causes is discussed.

8. The suggestion is made that certain types of incompatibility may be dependent on factors residing in the tissues of stock and scion in contact with each other at the

union rather than on the interaction of particular leaf and root systems. Possible explanations of the causes of incompatibility in the plants examined are given and future lines of work are suggested.

ACKNOWLEDGMENTS.

I wish to thank Dr. W. S. Rogers for providing facilities for this work to be carried out in the Pomology Section at East Malling Research Station.

The investigation was made under the general supervision of Dr. A. B. Beakbane to whom many thanks are due for her unfailing help throughout.

I am most grateful to Mr. R. J. Garner for giving me the benefit of his wide experience in grafting and general nursery problems ; to Miss B. G. Mosse for much help and criticism ; to Miss E. C. Thompson for the photographs and to Mr. J. Taylor for guidance on the statistical analysis.

Dr. R. D. Preston of the Botany Department, University of Leeds, has kindly given advice in connection with the studies on cambial activity.

To the authorities of La Junta de Relaciones Culturales, Madrid, my best thanks are due for granting the scholarship which made the present study possible.

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(*Received* 9/6/50.)

STUDIES ON INCOMPATIBILITY BETWEEN SOME PEAR AND QUINCE GRAFTS

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Although the use of intermediates to overcome incompatibility between stock and scion is now a common horticultural practice, the underlying principles are not at present fully understood. A better understanding of the function of the intermediate and of related problems of incompatibility is desirable, and it was thought that the construction of trees made up of two or more components grafted together in different combinations might prove a useful line of approach to the problem. The severe incompatibility observed in double-grafted trees of Conference pear on Quince A (intermediate), on C8, a vegetatively propagated pear rootstock, provided a particularly interesting starting point for such an investigation as the variety Conference made satisfactory four-year-old trees with good unions when single-grafted on either C8 (pear) or Quince A as a rootstock. As a result of this observation a small nursery trial was planted in 1945 to study the behaviour of trees made up of these three components grafted together in different combinations.

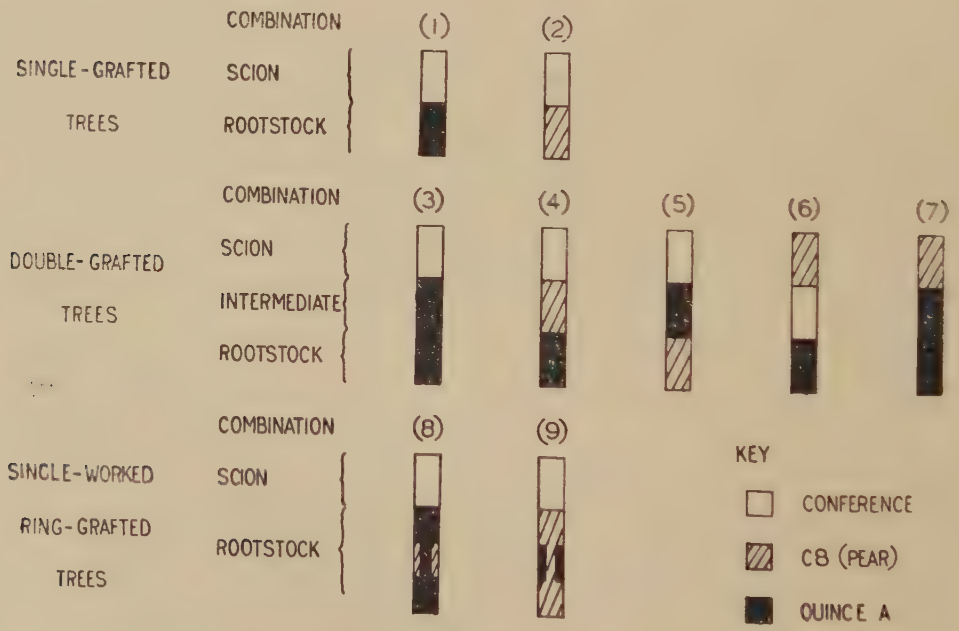
COMBINATION
(1)
(2)
SINGLE-GRAFTED
TREES
SCION
ROOTSTOCK
COMBINATION
(3)
(4)
(5)
(6)
(7)
DOUBLE-GRAFTED
TREES
SCION
INTERMEDIATE
ROOTSTOCK
COMBINATION
(8)
(9)
SINGLE-WORKED
RING-GRAFTED
TREES
SCION
ROOTSTOCK
KEY
☐ CONFERENCE
▨ C8 (PEAR)
■ QUINCE A

Fig. 1.

Plan of trees of Conference pear, Quince A and C8 pear grafted together in different combinations.

MATERIAL AND METHODS.

In addition to the double- and single-grafted trees of Conference, Quince A (QA), and C8 (pear) others were constructed which were worked higher than usual, and had a complete ring of bark 1 in. in width grafted into the stock stem, a form of veneer grafting described by Garner (1947), and usually referred to as a ring graft.

Trees constructed in the nine ways shown in Fig. 1 were available.

Both double- and single-worked trees were grafted in the spring of 1946, scions and intermediates being placed in position at the same time. The rootstock stems of some of the single-worked trees were ring-grafted some weeks later.

The trees were lifted in the spring of 1949 when the grafts were three years old. Records were made of weight and girth at lifting, and number of suckers and blossom trusses per tree. A description was made of the external appearance of the unions. They were then sawn in half longitudinally, smoothed off and a description made of the macroscopic appearance of the planed surfaces. The presence of starch in scions, intermediates and rootstocks was tested by treating free-hand sections with iodine. The observations on starch distribution are discussed below.

GROWTH AND APPEARANCE OF TREES.

The number of trees available, their mean weight and number of blossom trusses are shown in Table I, columns i-iii.

It must be pointed out that the results are based on data from small numbers of trees, only three years old, and not suitable for statistical analysis. Additional information available from fully grown trees of combination (1), Conf./QA, shows that such trees are healthy and fully compatible from a practical point of view. Although the weight of individual trees in each group varied considerably it was possible to recognize the most incompatible combinations with certainty by the small size of tree and the colour of the foliage. The double-worked trees of combination (5), Conf./QA/C8, and the ring-grafted trees of combinations (8), Conf./QA(C8 ring), and (9), Conf./C8 (QA ring), were obviously very incompatible. The trees had made very little growth, and some were nearly dead. They had few leaves, which were of a characteristic yellow-bronze colour, and they bore many blossom trusses for the amount of wood. Fig. 2 shows characteristic trees of combinations (2), Conf./C8, (3), Conf./QA/QA, (4), Conf./C8/QA, and (5), Conf./QA/C8, and illustrates the obviously unhealthy condition of trees of the latter combination. The unsatisfactory growth of trees of combination (5), Conf./QA/C8, and the striking effect of the Quince A intermediate were also observed in a previous nursery trial and are illustrated by Garner (1947). Trees of combination (4), Conf./C8/QA had made a normal amount of growth, and were unlike the controls (Conf./QA and Conf./QA/QA) chiefly in the larger number of blossom trusses. The marked difference in growth between trees of combinations (4), Conf./C8/QA, and (5), Conf./QA/C8, is of particular interest, as the only structural difference between them is the reversed position of intermediate and rootstock. This will be discussed later.

The ring-grafted trees of combinations (8), Conf./QA (C8 ring), and (9), Conf./C8 (QA ring), were very variable. They were dead or nearly so where the ring grafts remained unbroken, but where the rings had been split and bridges of stock tissue established the trees were recovering, and this was clearly shown in increased weight and vigour of individual trees. The tree of combination (7), C8/QA/QA, showed a type of incompatibility sometimes met with where growth is normal but the mechanical structure of the union is very weak.

The two cases, noted in Table I* where the root system was dead at the time of lifting are interesting as being definite instances of the death of the roots preceding that of the stem and branches. It is generally not easy to establish whether death occurs first in the roots or branches of incompatible trees.

The record made of the number of suckers indicated that there was a definite tendency to increased suckering from the rootstock in the incompatible trees.

Girth measurements of the stems of stock, scion and intermediate 5 cm. from the union gave only a poor indication of tree size, presumably due to the abnormal swelling near the union in some of the combinations.

STRUCTURE OF THE UNIONS.

Descriptions made of the unions of each tree as seen in longitudinal section showed that their structure within a combination was surprisingly uniform. They fell into fairly well-defined categories as follows :

Category A. Perfect unions.
The line of union in bark and wood was hardly visible.
The cut surfaces of the original grafts were in close contact, and there was no dying back at their extremities. Fig. 3(*a*).

Category B. Good unions.
The bark and wood were continuous although the line of union in the wood was often clearly distinguished by excessive ray formation and by distortion of the fibres.
The cut surfaces of the original grafts were separated by a layer of lignified callus, by ingrowing bark, or by a hollow space left as a result of disintegration of the callus tissue. The original grafts were frequently dying back at their extremities. Fig. 3(*b*).

Category C. Unions with discontinuous bark.
The appearance of the woody tissues of the union was frequently as described in Category B. The bark tissues of stock and scion were separated by a dark brown layer of corky appearance. Fig. 3(*c*).

Category D. Unions with discontinuous wood and bark.
The centre of the graft was frequently as in Category B. The woody tissues of stock and scion were separated in many places by groups of living, non-lignified parenchyma. Groups of parenchyma were nearly continuous in the last annual ring, and were beginning to form a complete layer of parenchymatous tissue. Bark tissue as in Category C (discontinuous). Fig. 3(*d*).

It is notable that of the fifteen unions examined between Conference and C8 (pear), twelve were perfect, and the rest good. In addition, two four-year-old unions of Conference on C8 (pear) examined in 1946 had also been very good. It seems reasonable to conclude that Conference makes a compatible union with the pear rootstock C8.

A point of general interest, shown in Table I, column (vii), was the superior quality of pear/pear over pear/quince unions, even where the tree as a whole appeared highly

TABLE I.

Field records and description of the unions of some trees of Conference pear, Quince A and C8 pear grafted together in different combinations.

No.	Combination.	(i) No. of trees examined.	(ii) Mean wt. per tree. lb.	(iii) Mean No. of blossom trusses.	(iv) Colour of foliage.	(v) Union.	(vi) Union : Description of External Appearance.	(vii) Union : † Internal structure (Macroscopic)
(1)	Conf./QA	1	0·8	0	green	Conf./QA	Variable union, one side was well joined, the other side was not growing much.	B
(2)	Conf./C8	2	1·6	0	green	Conf./C8	Very good union.	A
(3)	Conf./QA/QA	2	2·1	0·5	green	Conf./QA	Fairly good union : Conference was overgrowing QA slightly.	A-B
						QA/QA	Variable union : well joined on one side of the stem, but the union was bad on the other side where there was a large dead snag.	B
(4)	Conf./C8/QA	3	1·8	13	green	Conf./C8	Good union.	A-B
						C8/QA	Moderate union, C8 was overgrowing QA considerably.	D
(5)	Conf./QA/C8	5	0·4	5	yellow-bronze	Conf./QA	Fairly good union, Conference was tending to overgrow QA.	B
						QA/C8	Moderate union, the bark was badly joined in all cases.	D
(6)	C8/Conf./QA	6	2·6	0	green	C8/Conf.	Very good union.	A
						Conf./QA	Fairly good union, QA was much overgrown by Conference.	C
(7)	C8/QA/QA	1	2·7	0	green	C8/QA	Union broke off during lifting, leaving a smooth breakage surface. QA was slightly overgrown by C8.	D
						QA/QA	Variable union, good on the side where there was a very small vascular connection in the C8/QA union above, bad union on the other side.	B
(8)	Conf./QA (C8 ring)	6*	0·6	9	yellow-bronze	Conf./QA	Unions varying from moderate to very bad ; they were generally better above a bridge than above an unbroken ring. Conference was overgrowing QA.	C
						QA/C8 top of ring.	Bad unions.	D
						C8/QA base of ring.	Bad unions.	C
(9)	Conf./C8 (QA ring)	4*	0·75	1·5	yellow-bronze	Conf./C8	Unions varying from very good to moderate.	A-B
						C8/QA top of ring.	Bad unions	D
						QA/C8 base of ring.	Bad unions.	D

* In one tree the roots were dead while the stem above the ring was still alive.

† Macroscopic appearance of radial longitudinal sections of the unions is described in categories, column (vii):

Category A = perfect unions.
Category B = good unions.
Category C = unions with discontinuous bark.
Category D = unions with discontinuous wood and bark.

incompatible, as in combination (9), Conf./C8 (QA ring). Grafts between Conference and Quince A on the other hand were never in Category A (perfect), and sometimes in Category C (bark discontinuous). In combinations (6), Conf./QA/C8, and (8), Conf./QA (C8 ring), the type of union between Conference and Quince A had deteriorated to such an extent as to become discontinuous in the bark, a condition never observed in single-grafted trees of Conf./QA. In the pear/quince unions there was frequently a gap of up to 1 cm. between the original graft surfaces, making them appear as if they had been pulled apart forcibly, and causing the tongues of the whip and tongue grafts to be distorted and bent outwards in an unnatural position. It seems that the quality of unions between pears and quince is subject to modification depending on the general health of the tree, whereas pear/pear unions were found to be very good even where the trees were unhealthy.

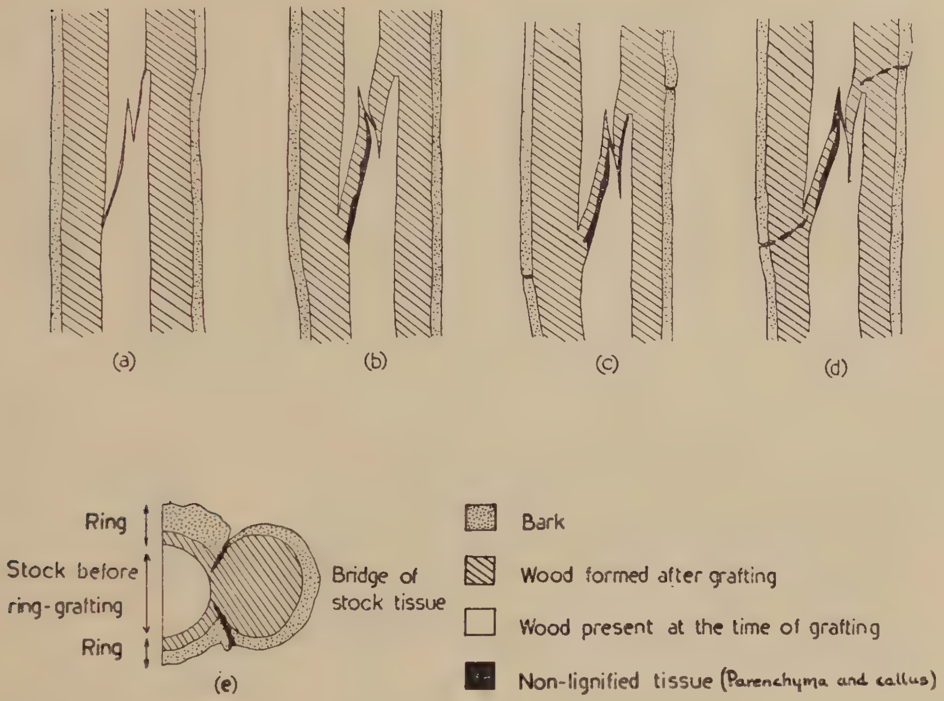
(a)
(b)
(c)
(d)
Ring
Stock before ring-grafting
Ring
Bridge of stock tissue
(e)
Bark
Wood formed after grafting
Wood present at the time of grafting
Non-lignified tissue (Parenchyma and callus)

FIG. 3.

Diagram showing different types of union observed. (a-d) longitudinal radial section, (e) transverse section.

The examination also showed that the structure of the unions in combination (5), Conf./QA/C8, was not appreciably worse than that in combination (4), Conf./C8/QA. This similarity is of special interest because the trees of the two combinations differed greatly in growth. While it is generally considered that badly joined discontinuous unions impede the passage of food substances between stock and scion (Chang, 1938), the different performance of the two combinations must in this case be due to some factor other than simple obstruction at the union.

Some comment should be made on the appearance of the ring-graft unions. In many of these there were longitudinal layers of living tissue (parenchyma) between the core of the stock stem and the newly-formed tissues of the ring graft. Fig. 3(*e*) shows the position of these layers of parenchymatous tissue frequently found between the bridging tissue and the broken graft ring. It is interesting that the scion was often better joined to the rootstock stem at a point just above the bridge than above a point where the graft ring was unbroken.

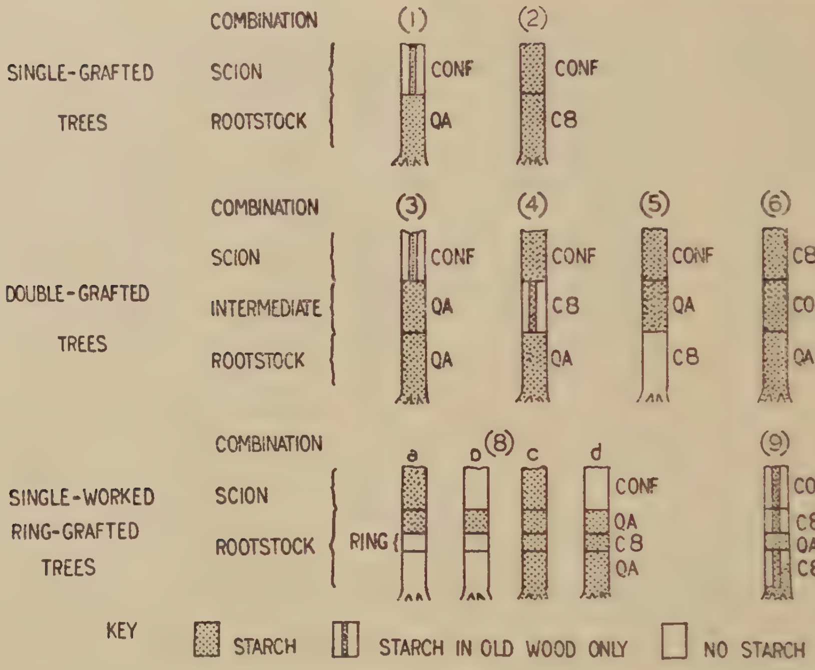
COMBINATION
(1)
(2)
SINGLE-GRAFTED TREES
SCION
ROOTSTOCK
CONF
QA
CONF
C8
COMBINATION
(3)
(4)
(5)
(6)
(7)
DOUBLE-GRAFTED TREES
SCION
INTERMEDIATE
ROOTSTOCK
CONF
QA
QA
CONF
C8
QA
CONF
QA
C8
C8
CONF
QA
C8
QA
QA
COMBINATION
a
b
(8)
c
d
(9)
SINGLE-WORKED RING-GRAFTED TREES
SCION
ROOTSTOCK
RING
CONF
QA
C8
QA
CONF
C8
QA
C8
KEY
STARCH
STARCH IN OLD WOOD ONLY
NO STARCH

Fig. 4.

Diagram of starch content of some trees of Conference pear, Quince A and C8 pear grafted together in different combinations.

In all unions examined the Quince A tissue invariably had a thinner bark than either Conference or C8 (pear).

Starch content of trees.

A survey was made of the starch content of different parts of each tree by placing freehand sections in a solution of iodine. Roots were included as well as parts of the stem. The results expressed diagrammatically in Fig. 4 illustrate the starch content of the wood only.

There was little starch in the bark, and none was ever found when there was no starch in the wood. In some trees starch was present only in the old wood, and where this occurred it is indicated in the diagram.

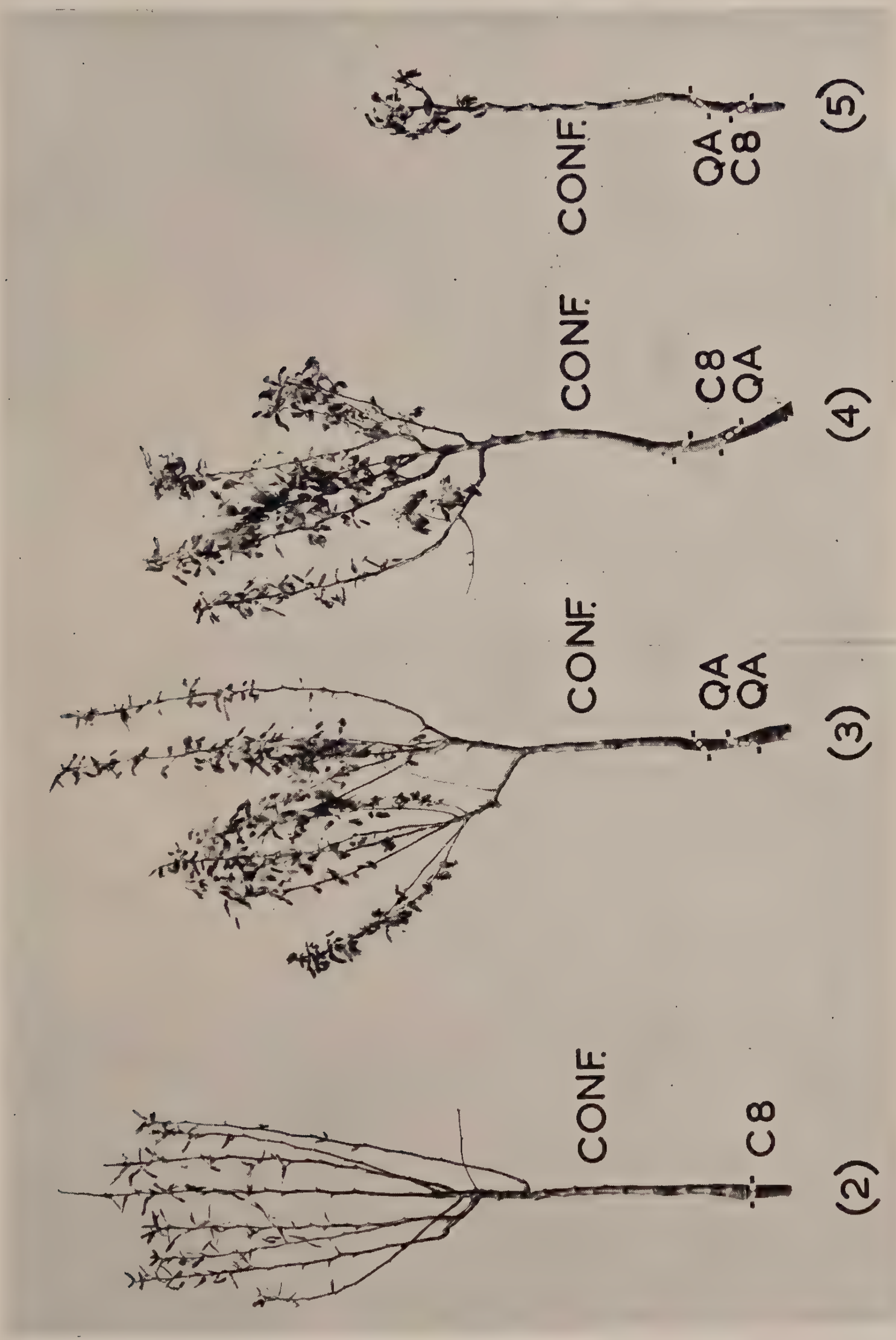

Fig. 2.

Typical three-year-old trees of combination (2), Conference/C8 (pear); (3), Conference/Quince A/Quince A; (4), Conference/C8 (pear)/Quince A; and (5), Conference/Quince A/C8 (pear).

N.B.—Trees of combination (2), Conference/C8 (pear), were lifted two weeks before the others and heeled in, and this accounts for the difference in the stage of development of their leaves.

In general, trees belonging to a particular combination showed similar distribution of starch in both stems and roots. Combinations (4), Conf./C8/QA, and (5), Conf./QA/C8, previously noted for their different behaviour notwithstanding the similarity in the structure of their unions, were of special interest. In both combinations the C8 tissue, constituting either the intermediate or the rootstock, was lacking in starch. Only when this lack of starch occurred in the rootstock was the resulting tree very poor.

In combination (8), Conf./QA (C8 ring), there was much variation in starch content between different trees. The tree (*c*) containing starch throughout had the largest bridge of stock tissue, and was growing most normally, while the tree with an unbroken ring-graft (*a*) contained no starch in or below the ring. Others were intermediate for both bridging and starch content, although there was not a clear correlation.

Where a rootstock without starch had suckers coming from the roots, a small part of the root system in direct connection with the sucker shoot contained some starch.

Although the abnormalities observed cannot be fully explained at present, it seems that the possible difficulties in the translocation of sugars due to a discontinuous union are not directly responsible for the absence of starch from the tissues below. This view is supported by the following observations :

(1) Unions showing lack of starch in the tissues below them were not always in Category D (discontinuous wood and bark).

(2) The lack of starch in the intermediate and its abundant presence in both scion and rootstock in combination (4), Conf./C8/QA, would be difficult to explain on the basis of impeded translocation.

(3) Fehling's test, applied to some of the material lacking starch indicated the presence of some reducing material, probably sugar, in all the parts tested. The presence of sugar shows that some translocation of food substances had taken place.

DISCUSSION.

One of the difficulties encountered in the study of incompatibility is the lack of definite symptoms. Our investigation showed that discontinuity of the tissues at the union was not always directly correlated with tree performance. Combinations (4), Conf./C8/QA, and (5), Conf./QA/C8, were of particular interest in this respect. While their unions were in approximately the same categories as regards their structure, starch content and tree performance differed considerably between the two combinations. Reversing the position of intermediate and rootstock had a profound effect on the tree, although the scion is compatible when single grafted on either as a stock. It would seem, however, that some kind of incompatibility exists between Quince A and C8 (pear) which caused severe symptoms of incompatibility such as lack of growth, yellow-bronze foliage, excessive fruit bud formation and discontinuity of vascular elements at the union, in trees where there was some QA tissue above C8, as in the combinations (5), Conf./QA/C8, (8), Conf./QA (C8 ring), and (9), Conf./C8 (QA ring). Where the orientation was reversed and the C8 tissue was above QA, as in combinations (4), Conf./C8/QA, and (7), C8/QA/QA, symptoms of incompatibility did not occur in the scion, but were confined to the mechanical structure of the unions themselves.

Differences in behaviour of the scion in reciprocal grafts are not unknown (Heppner and McCallum, 1927). They were also encountered in citrus by Toxopeus (1936), who came to the conclusion that they were due to the interaction between a particular leaf system and a particular stem or root system. In the present case such an hypothesis would not explain the different behaviour of combinations (4), Conf./C8/QA, and (5), Conf./QA/C8, since in these combinations the intermediates bore no leaves. Conference used as scion in both cases made satisfactory trees in this trial, in a previous nursery trial, and in a field trial now in its fifth year with both QA and C8 root systems.

Regarding the problem from a physiological point of view, it is not so surprising to find that reciprocal grafts may behave differently. Considering first the problem of translocation, it is generally accepted that water and inorganic substances move upwards in the plant, passing mainly through the wood, and it is unlikely that they undergo any major changes during their passage through this fairly inactive tissue. On the other hand elaborated food substances moving downwards pass mainly through the phloem, a highly active tissue, and are possibly subject to rapid changes. The problem of polarity may also be involved. Thus it is conceivable that the relative positions of the graft components of a tree are of importance, and that a latent incompatibility between two partners may express itself differently in reciprocal grafts. In one combination an apparently healthy though structurally weak tree may result, the inability of tissues to interlock at the union being a purely local reaction, whereas the reciprocal graft may exhibit signs of a much more serious physiological incompatibility which, however, does not necessarily cause a worse union. The results of the trial described in this paper could be explained on such an hypothesis.

The ring-grafted trees deserve some comment. It was possible to induce symptoms of severe incompatibility in normal single-worked trees by inserting a ring graft of a third variety in the stock stem. In this operation only the bark and the attached cambium of the third variety are transferred. The new tissue formed from the bark ring, without leaves or roots of its own variety, must have remained sufficiently distinct from the surrounding tissues to cause the reaction resulting in an incompatible tree.

In every case ring-grafted trees (with unbroken rings) were more incompatible than double-grafted trees having the same rootstock and scion and an intermediate of the ring-graft variety. A possible explanation is that when the ring graft becomes established the tree can be regarded as virtually triple-grafted and having three unions. All the ring-grafted trees would then have a piece of QA stem grafted on C8 tissue, and their behaviour could be explained if the suggestion, previously put forward, of a severe physiological incompatibility of QA on C8 is accepted.

This investigation gave an opportunity to compare graft unions of Conference on pear and quince stocks. In all cases the pear/pear unions were more nearly perfect than those of pear/quince, which were frequently associated with an apparent dislocation of the original grafts so that they were separated by lignified callus, parenchyma or hollow spaces. It seems likely that such symptoms, although frequently described as normal to all graft unions, should be regarded as signs of slight uncongeniality, which view is supported by the uneven starch distribution in trees of Conference on Quince A. Such mild forms of incompatibility may be insignificant from a practical point of view, and may even be useful in encouraging earlier cropping.

SUMMARY.

Single, double and "ring-grafted" trees were constructed to study the way in which two compatible graft components (Conference pear and Quince A) may react to the introduction, either as rootstock or intermediate, of a third variety (C8, pear) which made satisfactory trees with strong unions when single grafted with Conference. Records of growth and number of fruit buds were made on the three-year-old trees. The external appearance of the unions and the macroscopic appearance of longitudinal radial sections of the unions were described. Tests were made for the presence of starch.

(1) In all combinations examined Quince A tissue invariably had a thinner bark than either Conference or C8 (pear).

(2) Unions of Conference on C8 (pear) were mechanically more perfect than those of Conference on Quince A, which showed slight signs of uncongeniality.

(3) Poor tree performance was not always directly related to mechanically defective unions, but was frequently associated with abnormal starch distribution, particularly lack of starch in the root system.

(4) Symptoms usually associated with incompatible trees were produced in compatible single-grafted trees by the introduction of a bark ring of a third variety into the stock stem. Such symptoms were related to tree performance and/or structure of the graft union.

(5) Ring-grafted trees were more incompatible than double-grafted trees having the same rootstock and scion, and an intermediate of the ring-graft variety.

(6) Reversing the position of intermediate and rootstock greatly affected the performance of the Conference scion which made satisfactory trees when single-grafted on either as a rootstock. The combination Conference/Quince A/C8 and all the other combinations in which any tissue of QA occurred above C8 grew very badly, whereas the combination Conference/C8/QA grew well. Incompatibility in the former combinations could not have been due to an uncongenial leaf system, as has been found to be the case in other plants, but the relative positions of rootstock and intermediate appeared to control the degree of incompatibility of the tree.

ACKNOWLEDGMENTS.

The authors wish to express their thanks to Mr. R. J. Garner who made the first observation of incompatibility between Conference, Quince A and C8 (pear), and provided the material for this study. Thanks are also due to Dr. A. B. Beakbane for help and advice in the investigation.

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(*Received* 5/7/50.)

REVIEWS

BOTANY: AN INTRODUCTION TO PLANT SCIENCE. By W. W. Robbins and T. E. Weier. (John Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London, 1950, pp. ix + 480; 490 figs. Price 40s. net.)

The authors of this book are professors of botany at the College of Agriculture in the University of California, and we can be assured that they are highly competent to expound their particular science to the student who contemplates taking a course in Botany. As it is an introduction to Botany, specialist botanists will not expect to find any new information on their own particular subjects, though even they must admire the way in which the various aspects are brought together to form a compendium of our present-day knowledge of plant life and of recent botanical research. The general aim is indicated in the preface in these words:

"The authors have endeavoured to give the student a modest understanding of the scope of botany and its relation to agriculture, forestry, and medicine and to other physical sciences; to point out what botany has contributed to our knowledge of the world in which we live; to stress the all-important role of plants in the economy of nature; in short to arouse interest and to process the student's thinking so that he has a feeling that the course of study has significance to him and is related to his everyday life."

The book deals first (Chapter 1: Introduction) with the plant world in general showing the importance of plants to man's existence, emphasizing that agriculture is a basic industry. One section shows the interrelation of botany and other sciences (particularly chemistry) and another discusses what a botanist is and what he does.

In Chapter 2 is a brief review of the world's plants in relation to their distribution and their uses for providing food, for industrial purposes, and in medicine, with references to plants that provide spices and beverages, ornamental plants and those that are harmful, either directly to man because of their poisonous properties, or as weeds that lower the value of his crops.

Then follow chapters on types of vegetation, classification and naming of plants, their various organs and their functions, reproduction, genetics, and an account of the main divisions of the plant kingdom with illustrated descriptions of selected examples.

The fungi are dealt with at some length, in two chapters, particularly with reference to the losses they cause to crop plants. The viruses are described, in Chapter 18, with electron photographs, and with other illustrations to show some of the disease symptoms they cause in plants.

The student is introduced to hormones, vitamins, antibiotics, genes and polyploidy (but not heterosis), subjects absent from the botany text-books of the reviewer's early student days.

It is all written as simply as possible; botanical terms are not avoided since the student must become acquainted with them, but they are described clearly and, where relevant, illustrated. Perhaps this simplification is exaggerated sometimes, and we find "pith" and "pith rays" where "medulla" and "medullary rays" (not difficult words) would commonly be used.

A double-column format and the well-spaced type afford easy reading. The 490 illustrations have the parts clearly indicated, and a review of them will enable the student to revise quickly the salient points of the text, without necessarily referring to the text itself. Many of the illustrations have been reproduced from monographic treatises and their origin is acknowledged, but there is no " bibliography " to enable the student to see the scope (and possibly the price) of a book he may wish to see, or acquire, in order to study more closely a particular side line which interests him.

Some of the photomicrographs of the phases in nuclear division (Figs. 5.16 ; 5.18 ; 5.22) are not very clear and the young botanist may wonder how the stages so precisely set out in the diagrams can be deduced from such preparations.

There is a slip in the diagram showing the life cycle of the brown rot fungus (Fig. 17.15) ; the conidial stage shown is *Botrytis* ; it should be *Monilia*.

These defects, however, detract but little from the value of a book so admirably designed to enable the reader (be he student of botany, agriculture or horticulture, or the interested amateur) to understand what the study of botany means in the world to-day. Because of its size it is not a convenient handbook, and its price precludes its popularity among English students, but it will be a welcome addition to any botanical library to which students have ready access. H.W.

MONOGRAPHIE DES PRINCIPALES VARIÉTÉS DE PÊCHERS. (Monograph of the chief peach varieties.) By H. Caillavet and J. Souty. (La Maison Rustique, 26 Rue Jacob, Paris VI^{e}, 1950, pp. 416, illus., 11 by 8½ in.)

A renewed interest in peach-growing amongst English fruit growers was noticeable even before the appearance in 1947 of Mr. Justin Brooke's book, *Peach Orchards in England.* It is at least arguable that this revived interest is mainly due to a progressive climatic change, bringing appreciably warmer summers than were prevalent thirty or forty years ago. It may now be further stimulated by this magnificent work of two French pomologists on peach and nectarine varieties. It must be said at once, however, that even amongst the 363 varieties described by the authors, many well known in England are omitted. Whilst some ten varieties of English origin are included, and also some of French and American origin well-known in England, such as Grosse Mignonne Hâtive and Hale's Early, others, of much importance in English outdoor peach growing are not even mentioned, including both Peregrine and Rochester. The book will thus be of more interest to gardeners than to commercial growers.

But its prime interest will be to the systematic pomologist. The authors are unduly modest in describing their objects : " To establish methods of reliable identification ; to reduce to order the nomenclature of varieties, and thus give to those interested the possibility of obtaining a guarantee of trueness to name ; and finally to indicate the most important varieties. We do not pretend to do more." Yet they have certainly accomplished more than this.

The book might be said to mark a new step forward in methods of systematic pomology. The scope and completeness of the authors' system of classification is shown by their use of only 49 of the 168 possible groups into which they divide varieties. Their system is based, first, on downy and smooth skin (peaches and nectarines) ; secondly on colour of flesh—white, yellow, or red ; thirdly on flower shape (*not* size) ; fourthly on the free- and cling-stone character ; fifthly on the absence, or shape when present, of the petiole glands ; and sixthly on the colour of the flesh next the stone.

Their descriptions also are very complete. They include every character that might be useful for identification, and also notes over a ten year period on the susceptibility of each variety to certain diseases and to frost injury. The fifteen colour plates are particularly fine, showing, as do also the sixty additional black and white plates, the characters of foliage, shoot and blossom, fruit (in three aspects), and stone. Descriptions of all the varieties are also given in tabular form.

The classification makes possible, for the first time so far as the present reviewer knows, a large-scale analysis of variety characters and their correlation. The proportion of varieties with certain characters is, of course, much influenced by selection, and can have no genetic significance. Thus of the 363 varieties, nearly 89 per cent. are free-stone, obviously because free-stone varieties are preferred. A curious instance of correlation, which seems unlikely to be influenced by selection, appears in the classes based on flesh colour and flower shape ; in the white-fleshed class the two flower shapes occur in nearly equal numbers, whilst in the yellow-fleshed class over 80 per cent. have " campanulate " flowers. N.H.G.

FIELD EXPERIMENTS WITH CLONAL CACAO

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THREE field experiments with cacao that were planted at River Estate, Trinidad, B.W.I., between the years 1937 and 1940 were described by Cheesman (1941) and have since been the subject of progress reports (Cheesman, 1941; 1944; 1944-47). The first reasonable crops were borne in 1942 so that now data from six crops have accumulated. Their formal analyses are given here; these strengthen certain tentative conclusions regarding the relative merits of the selections under test and methods of propagation, drawn earlier from more limited data.

THE SPLIT-PLOT DESIGNS.

In each of the experiments CRB 1 and CRB 2, three treatment comparisons were originally included—between (1) selected clones, (2) methods of propagation and (3) kinds of planting material. Six clones are compared in each experiment, using ICS 1 as a standard clone common to both experiments. Each clone is propagated by buddings on seedling rootstocks and by cuttings. The third comparison is between the performance of trees propagated from "fan" material (cuttings and buddings) and that of trees propagated from "chupon" material (cuttings and buddings). These terms "fan" and "chupon" are applied to the two kinds of branches of a cacao tree (Cheesman, 1935). The former have indeterminate growth and their leaves are borne in two ranks, whereas the leaves of the latter are arranged in a $\frac{3}{8}$ spiral and growth is determinate. The stem of a young cacao seedling is a chupon and, ultimately, its terminal growing point is converted at a height of 3 or 4 ft. into a whorl of three, four or five spreading fan branches. Any bud developing on the main axis repeats this behaviour and gives in its turn a whorl of fan branches a few feet higher than the first. A third and fourth tier may be added to the tree in the same way. The tree that is produced from a cutting or budding of chupon material is similar in habit to a seedling. On the other hand, as higher order branches on fan material are similar fan branches, trees from fan cuttings or buddings lack a defined trunk, and have spreading branches that require pruning to make a symmetrical tree.

CRB 1 and CRB 2 each consists of 576 trees arranged in three replications of 192 trees. Halves of each replication are allotted at random to trees propagated from fan and chupon material respectively (i.e. 96 of each type). These half-replications are further divided into six plots (each of 16 trees) to which six different clones are assigned at random. Each 16-tree plot is then divided lengthwise in the E.-W. direction; and to the halves are allotted at random (*a*) trees propagated as cuttings and (*b*) trees propagated as buddings on seedling rootstocks. The final result is as shown in the plan of CRB 1 which compares clones ICS 1, 3, 4, 5, 6 and 8 (Fig. 1). CRB 2 compares, on precisely similar lines ICS 1, 7, 16, 22, 45 and 53.

In CRB3, also of 576 trees, the fan *v.* chupon comparison is omitted, so that this lay-out consists of six blocks of 96 trees. Each block contains six plots to which the clones are assigned at random and the plots are split, as before, for the cutting *v.* budding comparison. The six clones of CRB 3 are ICS 1, 2, 9, 12, 60 and 98.

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* Now at the Commonwealth Potato Collection, Cambridge, England.

All three CRB experiments carry shade trees of "Immortelle" (*Erythrina poeppigiana* (Walp.) O. F. Cook) at 24 ft. by 24 ft. spacing, to be thinned at a later date to 48 ft. by 48 ft. spacing. The cacao trees are planted at 12 ft. by 12 ft. spacing.

The experiments are established on River Estate loam, a soil type with good physical characteristics and displaying a well developed profile. There is an upper horizon, 12 in. to 15 in. in depth, consisting of dark brown loam or sandy-loam possessing a good crumbly structure. The reaction is slightly acid and the inherent fertility is medium, though showing deficiencies in phosphate and potash (especially the former); its organic content is medium.

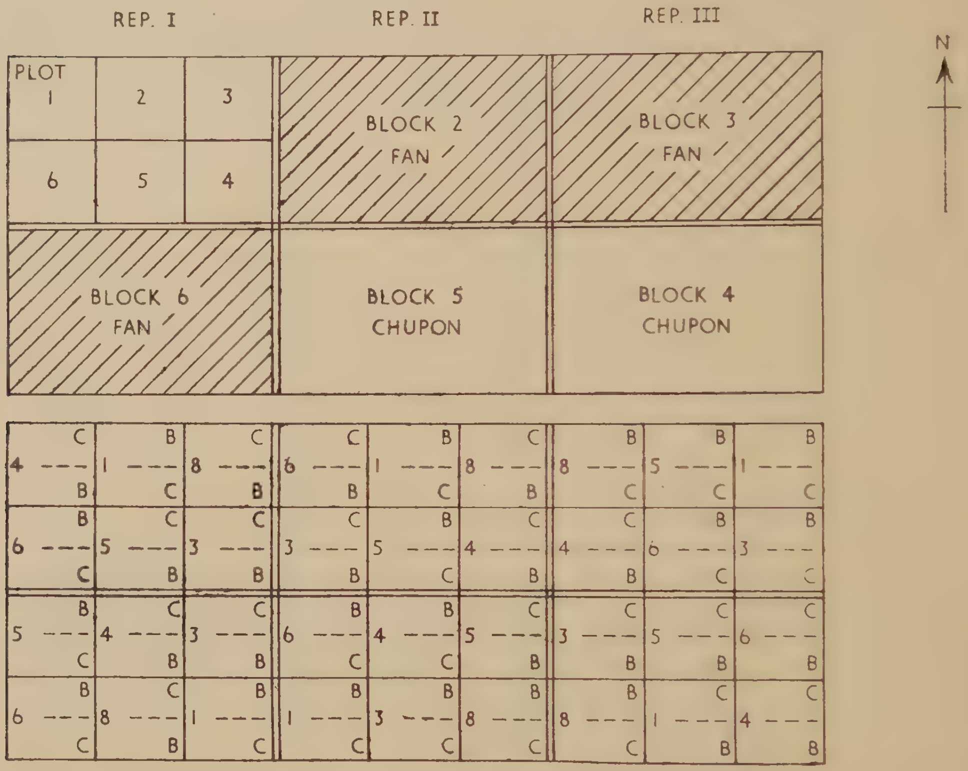
REP. I
REP. II
REP. III
N
PLOT 1
2
3
6
5
4
BLOCK 2 FAN
BLOCK 3 FAN
BLOCK 6 FAN
BLOCK 5 CHUPON
BLOCK 4 CHUPON
C 4 --- B
B 1 --- C
C 8 --- B
C 6 --- B
B 1 --- C
C 8 --- B
B 8 --- C
B 5 --- C
B 1 --- C
B 6 --- C
C 5 --- B
C 3 --- B
C 3 --- B
B 5 --- C
C 4 --- B
C 4 --- B
B 6 --- C
B 3 --- C
B 5 --- C
C 4 --- B
C 3 --- B
B 6 --- C
B 4 --- C
C 5 --- B
C 3 --- B
C 5 --- B
C 6 --- B
B 6 --- C
C 8 --- B
B 1 --- C
B 1 --- C
B 3 --- C
B 8 --- C
B 8 --- C
C 1 --- B
C 4 --- B

FIG. 1.

Layout of Experiment CRB 1 showing, above, the numbering of the replications, blocks and plots, with the allocation of blocks to fan and chupon material: and below, the distribution of the six clones and of sub-plots of buddings (B) and of cuttings (C).

In mid-June, 1947, a fourth treatment was incorporated in CRB 1 by dividing each 16-tree plot in the N.-S. direction, giving sub-plots containing four buddings and four cuttings each. A manurial treatment consisting of 100 lb. pen manure with 0·8 lb. muriate of potash and 1·5 lb. superphosphate per tree was allotted at random to one of each pair of sub-plots, the other being unmanured—giving 36 replications in all. Isolation trenches between the manurial sub-plots have been regularly opened and refilled.

At the same time, to similarly sub-divided plots in CRB 3, to each tree was made the first application of 100 lb. cut bush* with 0·8 lb. muriate of potash and 1·5 lb. superphosphate; the treatment was repeated at six-monthly intervals. Furthermore, three of the six 96-tree blocks (analogous with their use in CRB 1 and CRB 2 for the fan *v.* chupon comparison) received a spray treatment of "Hortomone A" (0·3 per cent. solution) at the rate of 2 gallons per tree. It was hoped that the growth substance would be taken up by the leaves and, in conjunction with the manurial treatment, exert a beneficial effect on the trees.

EXPERIMENTAL DATA.

The data from CRB 2, 1 and 3 are presented in the order named.

Experiment CRB 2. This trial was planted in 1938 and its present composition with regard to age of trees is fairly homogeneous; of the 576 trees in the experiment, 561 are either eight or nine years old.

Fan material has given a mean yield of 12·75 kg. (*s.e.*=0·74) of wet cacao for an experimental unit of eight trees, and chupon material one of 13·05 kg. (*s.e*=0·74). This difference is not significant (P>·05); the experiment, however, scarcely makes a satisfactory test of this point as there are only two degrees of freedom for error. A far more efficient design would have been obtained by using 36 16-tree plots, arranged in three blocks of 12 plots, each plot receiving at random one of the twelve combinations of clones and kinds of planting material.

The clonal yields of fan and chupon material are given in Table I, and it is of interest to note that chupons of ICS 45 yielded significantly better than the corresponding fans. No significant yield differences are found amongst the other clones.

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* A mixture of coarse grass, herbaceous and semi-woody material.

TABLE I.

Experiment CRB 2. Yields of wet cacao (in kg.) for experimental units of 8 trees.

Clones.	Fan *v.* Chupon.		Buddings *v.* Cuttings.		Seasons.					
					1942-'43.	'43-'44.	'44-'45.	'45-'46.	'46-'47.	'47-'48.
	1·31†		1·17		1·86					
ICS 1	18·97	19·71	18·29	20·39	17·92	9·87	28·15	20·34	14·39	25·37
ICS 16	17·53	15·03	16·04	16·52	12·53	9·85	27·66	18·45	10·99	18·21
ICS 45	14·14	17·74	14·23	17·64	16·61	9·27	25·68	12·41	8·24	23·43
Means	16·88	17·49	16·19	18·18	15·69	9·66	27·16	17·07	11·21	22·34
	2·27		1·70		2·27					
ICS 53	10·94	10·56	10·12	11·38	8·92	6·50	21·66	9·90	5·67	11·85
ICS 22	8·71	9·64	7·68	10·66	9·13	4·87	12·12	9·35	6·59	12·96
ICS 7	6·21	5·60	6·80	5·01	1·16	3·61	10·48	9·07	4·53	6·58
Means	8·62	8·60	8·20	9·02	6·40	4·99	14·75	9·44	5·60	10·46

† Standard error of difference of two means appropriate to the entries appearing immediately below.

The clones fall into two yield groups ; three, ICS 1, 16 and 45, are good yielders, and the remainder, ICS 53, 22 and 7, are poor yielders (Table I). There are significant differences in yield between some of the members within each group : it may be noted, for example, that ICS 16 and 45 gave significantly lower yields than ICS 1 but there is no significant difference between the yields of ICS 16 and 45. ICS 7 is significantly poorer than either ICS 53 or 22.

The yields of these two groups are so markedly different that heterogeneity of variance might be expected ; accordingly the five degrees of freedom and sum of squares for clones were orthogonally partitioned into three parts : (1) one degree of freedom for the good *versus* poor comparison, (2) two degrees of freedom for contrasts within the three good clones and (3) two degrees of freedom within poor. Similar partitions were made in the interactions of clones with all other main effects except seasons. Appropriate partition elements of error variances were used for tests of significance and the calculation of standard errors.

The three best yielders have together given a mean annual yield of 573·1 lb. of dry cacao to the acre. A comparable figure for the yield that might be expected from unselected material of similar age and growth conditions cannot be given but, in the past, between 250 and 300 lb. of dry cacao to the acre has been obtained at River Estate as an annual average from the first six crops of seedling trees.

Cuttings have given a significantly higher yield than buddings ; the former have yielded 13·60 kg. (*s.e.*=0·32) of wet cacao per unit of eight trees and the latter 12·19 kg. (*s.e.*=0·32). Nevertheless, when clones are considered separately, only for ICS 45 and 22 are the yields of the cuttings significantly greater than those of the buddings ; indeed, in ICS 7 buddings have out-yielded cuttings, though the difference is not significant (Table I). This variability in the behaviour of clones as buddings or cuttings was highly significant, but only among the three low yielding clones. Either as buddings or as cuttings, ICS 1, 16 and 45 are significantly better yielders than ICS 53, 22 and 7. At the same time it must be noted that the cuttings of ICS 1 are outstandingly good in this series of clonal comparisons.

Significantly varying yields have occurred during the seasons (Table I), those for the three crop years 1944-45, 1945-46 and 1946-47 being progressively lower, with the end result that the yield of the 1946-47 crop was less than that of the first, 1942-43. In other words, at seven years of age the trees gave a lighter crop than at three years of age. The yield in the 1947-48 season, however, was good. In a series of favourable seasons, a progressive increase in yield might be expected from maturing trees. A small yield in the first crop season is usual but, clearly, the anomalous yields of the second and fifth seasons require explanation. Discussion on this point is deferred until the data from the other two experiments have been presented.

In every year ICS 1 occupied first place, though in some seasons its superiority was not as strongly marked as in others ; in like manner, the consistent inferiority of ICS 7 fluctuated ; nor was the order of merit of the other four clones the same every season. In each year ICS 1, 16 and 45 were the best three yielders, but the contrast between good clones together and poor is variable.

From Table II it will be seen that cuttings have out-yielded buddings in five of the six years, buddings giving a slightly higher yield in 1946-47. There appears to be a progressive reduction in the contrast between the yields of buddings and cuttings and this suggests that the initial difference between the two may not be maintained. This is made more evident in Table II when the seasonal yields of wet

TABLE II.

Experiment CRB 2. Yields of wet cacao (in kg.) of buddings and cuttings of each clone.

		1942-'43.	1943-'44.	1944-'45.	1945-'46.	1946-'47.	1947-'48.
ICS 1	B	14·83	9·76	26·80	19·85	12·86	25·63
	C	21·01	9·98	29·51	20·84	15·91	25·10
ICS 16	B	12·08	9·16	25·32	17·60	12·94	19·12
	C	12·97	10·54	30·01	19·30	9·04	17·30
ICS 45	B	14·46	8·48	22·82	11·83	7·35	20·46
	C	18·76	10·06	28·54	12·99	9·12	26·39
ICS 53	B	6·21	5·15	20·83	10·72	5·75	12·04
	C	11·63	7·85	22·48	9·08	5·59	11·67
ICS 22	B	6·09	3·80	10·19	8·53	5·92	11·58
	C	12·17	5·95	14·05	10·16	7·27	14·35
ICS 7	B	0·92	3·74	12·28	9·88	5·93	8·07
	C	1·39	3·49	8·68	8·26	3·13	5·08
Means	B	9·10	6·68	19·71	13·07	8·46	16·15
	C	12·99	7·98	22·21	13·44	8·34	16·65

B=budding, C=cutting; 8 trees of each.
Standard error of difference between cutting/budding means (last row) in different seasons=1·08.

cacao for the buddings and cuttings of each clone are examined. In the last two seasons, no less than half the entries show a reversal in favour of buddings.

Experiment CRB 1. This experiment was planted in 1937. The supplies (replanted trees) of 1939 were fairly evenly distributed amongst the clones and since that year, with the exception of ICS 5 (which is heavily attacked by the cocoa beetle, *Stirastoma depressum*), there has been a practically complete stand of trees. Hence, of the 576 trees in the experiment, 553 are either 8, 9 or 10 years old.

Fan material has given a seasonal mean yield of 12·39 kg. (*s.e.*=0·56) of wet cacao on a sub-plot of eight trees, and chupon material one of 10·03 kg. (*s.e.*=0·56), but the difference is not significant; as stated earlier, the very limited number of error degrees of freedom provides an unsatisfactory test of this point.

The clones fall into two yield groups. Three, ICS 1, 6 and 8, are good and three, ICS 4, 5 and 3, are poor; there are no significant differences within either group (Table III). The single degree of freedom provided by the contrast between the means of the good and poor clones accounts for the major portion of the sum of squares for clones.

On the assumption that 1 kilogram of wet cacao gives 0·88 lb. (400 gm.). of dry cacao, ICS 1, 6 and 8 together (288 trees covering 0·95 acre) have given an average of 1·98 lb. per tree of dry cacao or at the rate of 598 lb. of dry cacao to the acre per year.

With a mean yield of 13·01 kg. (*s.e.*=0·28) of wet cacao per sub-plot of eight trees, cuttings have given a significantly greater yield than buddings, of which the mean yield was 9·41 kg. (*s.e.*=0·28). Cuttings of ICS 1, 6, 8 and 4 have each

TABLE III.

Experiment CRB 1. Yields of wet cacao (in kg.) for experimental units of 8 trees.

Clones.	Buddings *v.* Cuttings.		Seasons.				
			1942-'43.	'43-'44.	'44-'45.	'45-'46.	'46-'47.
	1·79*		2·67				
ICS 1	16·44	20·84	19·37	22·21	26·89	14·16	10·56
ICS 6	15·05	20·79	14·10	20·64	19·07	26·52	9·29
ICS 8	13·80	21·12	13·01	23·93	24·25	18·27	7·85
Means	15·10	20·92	15·49	22·26	23·40	19·65	9·23
	0·73		2·17				
ICS 4	5·14	7·35	2·26	7·82	8·94	8·65	3·56
ICS 5	3·21	4·35	1·02	2·62	7·02	5·60	2·65
ICS 3	2·81	3·63	1·08	3·47	6·30	4·46	0·79
Means	3·72	5·11	1·45	4·64	7·42	6·24	2·33

* See footnote Table I.

given significantly greater yields than the corresponding buddings (Table III). In ICS 3 and 5, also, the cuttings have out-yielded the buddings, but the differences are not significant. ICS 1, 6 and 8 either as cuttings or as buddings have given significantly higher yields than the corresponding material of ICS 4, 5 and 3. There are no significant differences in yield between cuttings or between buddings within the three good clones, but in the three poor clones cuttings of ICS 4 are significantly better than those of ICS 3.

Seasonal yields have varied significantly and it will be observed from Table III, with the exception of ICS 6, that the last two crops have been progressively smaller. In the high yielding clones the crop from the fifth season is smaller than the first, though not significantly so. An exactly similar trend was noted above, in CRB 2 : in that experiment, a marked recovery in 1947-48 was observed and, in CRB 1, the yields of the unmanured plots for the 1947-48 crop show an equally good recovery from the low yields of 1946-47.

Table IV gives the clonal yields over the seasons and shows that their relative performance varied from season to season. This highly significant seasonal variation becomes more strongly marked when the good and poor clones are grouped—good clones tend to be more uniform in their seasonal behaviour (a range of 152 per cent. of the lowest seasonal yield), while poor clones fluctuate widely from season to season (a range of 413 per cent. of the lowest yield).

Over the seasons, fan material consistently and markedly out-yielded chupon material in the good clones ; whereas in the poor clones, chupon material actually out-yielded fan material by small amounts in three seasons out of five.

Cuttings were superior to buddings in all but the last (1946-47) season. It was observed in CRB 2 that the superiority in yield of cuttings was becoming less marked as the trees matured. A similar trend is not so apparent in CRB 1 (Table IV).

When the clonal yields of cuttings and buddings are examined over the seasons (Table IV), it is found that the relative magnitudes of the differences between the yields of cuttings and buddings in the good and the poor clones are fairly consistent.

Table IV.

Experiment CRB 1. Yields of wet cacao (in kg.) of buddings and cuttings of each clone.

		1942-'43.	1943-'44.	1944-'45.	1945-'46.	1946-'47.
ICS 1	B	18·29	19·62	23·41	11·73	9·15
	C	20·45	24·80	30·38	16·58	11·98
ICS 6	B	10·90	19·06	16·36	19·22	9·73
	C	17·30	22·23	21·79	33·81	8·85
ICS 8	B	9·01	17·92	18·48	15·93	7·66
	C	17·01	29·94	30·01	20·61	8·04
ICS 4	B	1·33	5·95	6·92	7·80	3·72
	C	3·20	9·69	10·96	9·49	3·40
ICS 5	B	0·72	2·16	6·13	4·36	2·67
	C	1·33	3·07	7·91	6·83	2·62
ICS 3	B	0·94	2·37	6·42	3·59	0·73
	C	1·23	4·58	6·18	5·34	0·84
Means	B	6·87	11·18	12·95	10·44	5·61
	C	10·09	15·72	17·87	15·44	5·96

B=budding, C=cutting; 8 trees of each.
Standard error of difference between cutting/budding means (last row) in different seasons=1·16.

Experiment CRB 3. This trial does not include the fan *versus* chupon comparison. It was planted in 1939 and compares the six clones ICS 1, 2, 9, 12, 60 and 98 as fan buddings and cuttings. Of the 576 trees, 518 are either 6 or 7 years old. The material of ICS 2 and 12 is rather heterogeneous for age.

Once again, the clones fall into yield groups. The degree of freedom provided by the contrast of the means of good clones (ICS 1, 60 and 98) and poor (ICS 2, 9 and 12) accounts for the major portion of the sum of squares for clones. The sum of squares provided by contrasts within the good group is highly significant and this may be related to the somewhat intermediate position occupied by ICS 98, which is significantly poorer than either ICS 1 or 60, the two best yielders. Exactly the same order of merit occurs when only cuttings of the good clones are compared. ICS 2, 9 and 12 are poor yielders, with ICS 2 significantly better than ICS 9 and 12, and on the basis of buddings alone, ICS 2 moves from the poor class to the mediocre class with ICS 98 (Table V).

Experimental units of eight trees have given mean yields of 11·36 kg. (*s.e.*=0·16) and 9·64 kg. (*s.e.*=0·16) of wet cacao as cuttings and buddings, respectively; this difference is significant. Within the clones, however, only cuttings of ICS 1 and 60 have given significantly better yields than buddings. In ICS 9 and 12 cuttings have yielded more than buddings but the differences are without significance, and ICS 2 buddings have actually yielded significantly better than its cuttings.

As might be anticipated from the two experiments already discussed, the effects of the seasons are significant in this experiment also. Yields rose progressively from the first to the penultimate crop but a reduction of yield occurred in the 1946-47 crop season. The order of merit of the clones within seasons is that among the good clones ICS 98 consistently ranks third, whereas ICS 1 and 60 often interchange

TABLE V.

Experiment CRB 3. Yields of wet cacao (in kg.) for experimental units of 8 trees.

Clones.	Buddings *v.* Cuttings.				Seasons.		
			1942-'43.	'43-'44.	'44-'45.	'45-'46.	'46-'47.
	1·42*		2·39				
ICS 60	14·49	18·98	10·43	19·90	19·62	23·23	10·51
ICS 1	13·57	18·78	10·70	15·67	16·78	20·86	16·86
ICS 98	10·55	13·77	7·49	15·56	13·82	14·73	9·20
Means	12·87	17·18	9·54	17·04	16·74	19·61	12·19
	0·78		2·16				
ICS 2	8·74	5·23	4·47	4·91	8·16	10·25	7·04
ICS 12	5·45	5·75	5·82	5·22	6·40	7·00	3·56
ICS 9	5·05	5·65	3·02	5·19	6·39	8·69	3·46
Means	6·40	5·54	4·47	5·11	6·98	8·65	4·69

* See footnote Table I.

first and second places; no consistency of ranking is observed amongst the three poor clones. The relative superiority of the good clones over the poor fluctuates rather widely from season to season as can be deduced from Table V.

As with CRB 2, the better yielding of cuttings over buddings shows a tendency to decrease as the trees mature (Table VI).

TABLE VI.

Experiment CRB 3. Yields of wet cacao (in kg.) of buddings and cuttings of each clone.

		1942-'43.	1943-'44.	1944-'45.	1945-'46.	1946-'47.
ICS 60	B	6·71	16·86	17·85	20·74	10·31
	C	14·15	22·93	21·40	25·71	10·70
ICS 1	B	7·21	12·87	13·60	19·19	14·99
	C	14·20	18·48	19·97	22·53	18·74
ICS 98	B	4·44	12·36	11·04	13·84	11·05
	C	10·51	18·76	16·60	15·63	7·34
ICS 2	B	3·36	6·92	10·34	14·65	8·44
	C	5·78	2·91	5·97	5·86	5·63
ICS 12	B	5·67	4·46	7·04	6·16	3·91
	C	5·97	5·99	5·76	7·84	3·21
ICS 9	B	2·55	5·08	5·19	8·56	3·85
	C	3·48	5·29	7·60	8·82	3·07
Means	B	4·99	9·76	10·84	13·86	8·76
	C	9·02	12·39	12·88	14·40	8·12

B=budding, C=cutting; 8 trees of each.
Standard error of difference between cutting/budding means (last row) in different seasons=1·28.

Experimental data from manurial treatments, 1947-48.

As mentioned above in the description of these experiments, manurial treatments were incorporated in CRB 1 and CRB 3, beginning in mid-June, 1947. The data for the 1947-48 crop are presented in terms of numbers of pods, these being the only individual tree records available.

CRB 1. Pen manure, fortified with potash and superphosphate fertilizers, was applied to sub-plots each containing four cuttings and four buddings. With clonal yields expressed in numbers of pods, ICS 4, which bears relatively large numbers of small pods, moves into the class of good bearing clones. As in previous analyses, all main effects and interactions of clones (including components of error) are orthogonally partitioned into three portions. Although cuttings out-yielded buddings in this experiment, the difference is not significant.

Manuring resulted in a highly significant increase in yield. The relative increase is less (37 per cent.) in the four good clones than in the two poor clones (66 per cent.); indeed, there is a significant negative correlation ($r = -0\cdot843$) between the percentage increase in yields and unmanured yields in the six clones (Table VII).

Table VII.

Experiment CRB 1. Yields, in numbers of pods from experimental units of 8 trees.

	ICS 1	ICS 4	ICS 6	ICS 8	ICS 3	ICS 5	Means
Manured	184·5	122·7	167·3	183·0	33·8	62·2	125·6
v. Unmanured	148·5	93·8	115·1	122·0	20·3	37·5	89·6
% increase over unmanured	24	31	45	50	67	66	

Standard error for differences within clones=9·94; for means (last column)=4·07.

Buddings and cuttings reacted almost exactly similarly to the application of manures (39·7 per cent. and 40·7 per cent. increases respectively).

Chupon material appears to benefit more from the application of manures than does fan material (increases of 45·2 per cent. and 35·5 per cent. respectively) but the differences in response are not significant.

The three replications show marked differences in their responses to manuring. Replication I, occupying the western end of the experiment, shows an increase of 67·2 per cent. in the manured trees over the unmanured; in replication II, occupying the middle section of the experiment, the increase is 46·8 per cent., whilst replication III, at the eastern end of the experimental area, shows only 11·1 per cent. increase.

CRB 3. "Hortomone A" spray was applied to three of the six blocks; and cut bush, fortified with potash and superphosphate fertilizers, was applied to sub-plots each of four cuttings and four buddings. With clonal yields expressed in numbers of pods, no obvious grouping into good and poor yielders is apparent and for that reason the five degrees of freedom for clones have not been partitioned in this experiment.

The application of a spray of " Hortomone A " to the foliage of the experimental trees is associated with an overall reduction in yield of 8 per cent., which is not, however, statistically significant. Clonal responses to the spray treatment vary rather widely, with the yield of ICS 60 lower by no less than 24·6 per cent. and with the yield of ICS 2 higher by 5·3 per cent. (Table VIII).

TABLE VIII.

Experiment CRB 3. Yields, in numbers of pods, from experimental units of 8 trees.

Clones.	Sprayed *v.* Unsprayed.		Manured *v.* Unmanured.	
			17·8*	
ICS 1	199·0	210·5	226·2	183·3
ICS 2	149·2	141·7	157·5	133·3
ICS 9	173·7	174·2	200·5	147·3
ICS 12	75·5	98·2	90·7	83·0
ICS 60	165·0	218·8	216·7	167·2
ICS 98	254·4	261·7	248·8	267·3
Means	169·5	184·2	190·1	163·6

* See footnote, Table I.

Cuttings as a whole out-yielded buddings, but not significantly so. The variability in the behaviour of clones as buddings or cuttings was highly significant owing to the marked superiority of ICS 2 buddings over its cuttings.

Trees given the mulch-cum-fertilizer treatment significantly out-yielded the untreated trees by 16·2 per cent. (Table VIII). Clonal responses to the manurial treatment vary considerably and, as in CRB 1, the best yielding clone was least benefited by the application of manure—indeed, the yield of ICS 98 is slightly lower when manured. Unlike CRB 1, however, the poorest yielding clone (ICS 12) does not show the greatest relative response—this is shown by ICS 60.

The reduction in yield with the " Hortomone A " spray treatment is less marked on plots receiving manures than on unmanured plots (4·5 per cent. and 11·9 per cent. respectively). This interaction is not, however, significant.

The yields of buddings appear to have increased more with manuring than have those of cuttings (18·8 per cent. and 13·7 per cent. increases respectively) but the difference is not significant.

DISCUSSION.

In each of these three experiments, the clones have fallen into yield groups, either good or poor. Of the seven good clones, ICS 1, 6, 8, 16, 45, 60 and 98, all except ICS 16 and 60 are self-fertile ; and of the nine poor clones, ICS 2, 3, 4, 5, 7, 9, 12, 22 and 53, all except ICS 7 and 9 are self-sterile. These proportions seem to suggest that a self-fertile clone is more likely to come within the class of good bearers than a self-sterile, although the probability calculated from χ^2 fails to attain significance. In view of the paucity of information on the breeding system of cacao, it is impossible at the present juncture to decide whether the yields of self-sterile trees are lowered as a result of inadequate pollination. Cope (1939), using 5-year-old seedling trees in close vicinity to these experiments, found that self-fertile trees yielded 72 per cent. more pods than self-sterile trees in the same population. The magnitude of the difference between the yields of self-fertile and self-sterile seedling trees is, however, considerably less than that between good and poor clones in the three CRB experiments, suggesting that genetic factors may largely be responsible for the low yields of the poor clones.

Owing to the relatively short duration of these trials in comparison with the life of a cacao tree, definite conclusions cannot be made concerning either (*a*) kinds of plant material (fan and chupon) or (*b*) methods of propagation (buddings and cuttings). Nevertheless, the data from the three experiments seem to indicate that (1) with the possible exception of ICS 45, there are no intrinsic differences in the yield potentialities of fan and chupon material and (2) a clone propagated on its own roots (cuttings) usually out-yields that clone on unselected seedling rootstocks (buddings) ; this is especially so with clones classified as good. This latter observation suggests that the good clones have root systems more efficient than those of the average run of seedlings, whereas those of the poor clones are less efficient ; the poor-yielding clone, ICS 2, is markedly improved by budding on to seedling rootstocks.

The magnitudes of the differences between the yields of cuttings and buddings, over successive seasons, vary quite considerably and on the whole, there appears to be a tendency for the yields from the two classes of tree to become more similar with lapse of time. This observation, unfortunately, is the outcome of a combination of two factors—the purely environmental one of season and the purely physiological one of maturation. During the period of the experiments, some seasons have been notably unfavourable to good cropping and it may well be that these poor yields have resulted not only from purely seasonal effects but also from physiological exhaustion of the young trees after heavy cropping in the preceding good season. This would be especially true of cuttings. With the accumulation of further crop records, it may be possible to judge the relative effects of seasons, age and previous performance on cropping.

SUMMARY

Analyses of records of yields of wet cacao are presented for three sets of clonal trials, CRB 1, 2 and 3, for five, six and five crop seasons respectively.

Each trial compares six clones. CRB 1 contains clones ICS 1, 3, 4, 5, 6 and 8 as fan and chupon material and as buddings and cuttings ; CRB 2 compares clones ICS 1, 7, 16, 22, 45 and 53 in the same way ; CRB 3 contains clones ICS 1, 2, 9, 12, 60 and 98 as buddings and cuttings only.

Clones ICS 1, 6, 8, 16, 45, 60 and 98, in their respective experiments, have given consistently good yields, whilst the remainder have performed poorly.

Except for ICS 45, the yields of fan and chupon material do not show any marked differences.

Cuttings have consistently out-yielded buddings, the superiority being most marked in the seven good clones.

Pen manure, augmented by artificial fertilizers, and cut bush, similarly augmented, applied in CRB 1 and CRB 3 respectively, in the sixth crop season, have given significant and favourable responses.

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(*Received* 12/1/50.)

A STUDY OF PLOT-SIZE WITH NIGERIAN ESTATE CACAO

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SYNOPSIS.

Crop records are examined from Nigerian native small holdings of cacao to find out how best such trees can be used as experimental material.

INTRODUCTION.

THE following paper describes a study of the variation in yield of cacao trees on certain native holdings at Agodi, near Ibadan, Nigeria. Six years' records of pod numbers on individual trees had been made by Mr. T. A. Russell of the Department of Agriculture, Ibadan, and, at his request, the writers have examined his records with a view to recommending a suitable technique for conducting simple field trials on such holdings.

The writers are indebted to Mr. Russell for the following description. The trees form in all a ten-acre block, divided into 27 holdings of varying shapes and sizes, some planted by different farmers, others by the same farmer but at different times. A few figures will show the diversity of the fifteen holdings that were available for study. In area they varied from 0·074 acres to 0·939 and in density from 322 trees per acre to 1,351. The oldest had been planted about 1918 and the youngest about 1935, though these dates are no more than rough estimates.

Despite their diversity these holdings are all roughly typical of the district, the trees being yellow-podded Amelonado grown with the aid of only a few shade trees and spaced irregularly without rows. The soil is a brown loamy clay overlying deep red clay of a kind generally considered suitable for cacao. The mean annual rainfall (1939-48) of 44·9 in. is low for the crop, but typical of a large part of the Nigerian cacao area round Ibadan.

The data supplied all represented annual crops in number of pods for the periods March to February inclusive. As will be explained, these were grouped to give crops in two-year periods.

In contrast to the trees of Cheesman and Pound [1] these were not regularly spaced, while they differed from those of Jolly [3] in forming smaller fields. Indeed, the largest containing only 0·939 acre, it would not have been practicable to have followed Jolly's recommendation [3] to use plots of 0·05 acre in a field of two or more acres. (These two previous studies of the variation of cacao had been carried out in the West Indies and the results could only be applied tentatively in Nigeria.) The problem was thus to discover how to use this land available at Agodi to the best advantage for experimental purposes, taking into consideration accuracy and economy of ground and material. In this study, use was made of methods of examining uniformity trial data recently described and applied to vines [4] and apple trees [6].

Alternatives were the use of (*a*) standard areas or (*b*) individual trees as plots. The latter was rejected for the reasons given by Jolly [3], namely, the difficulties

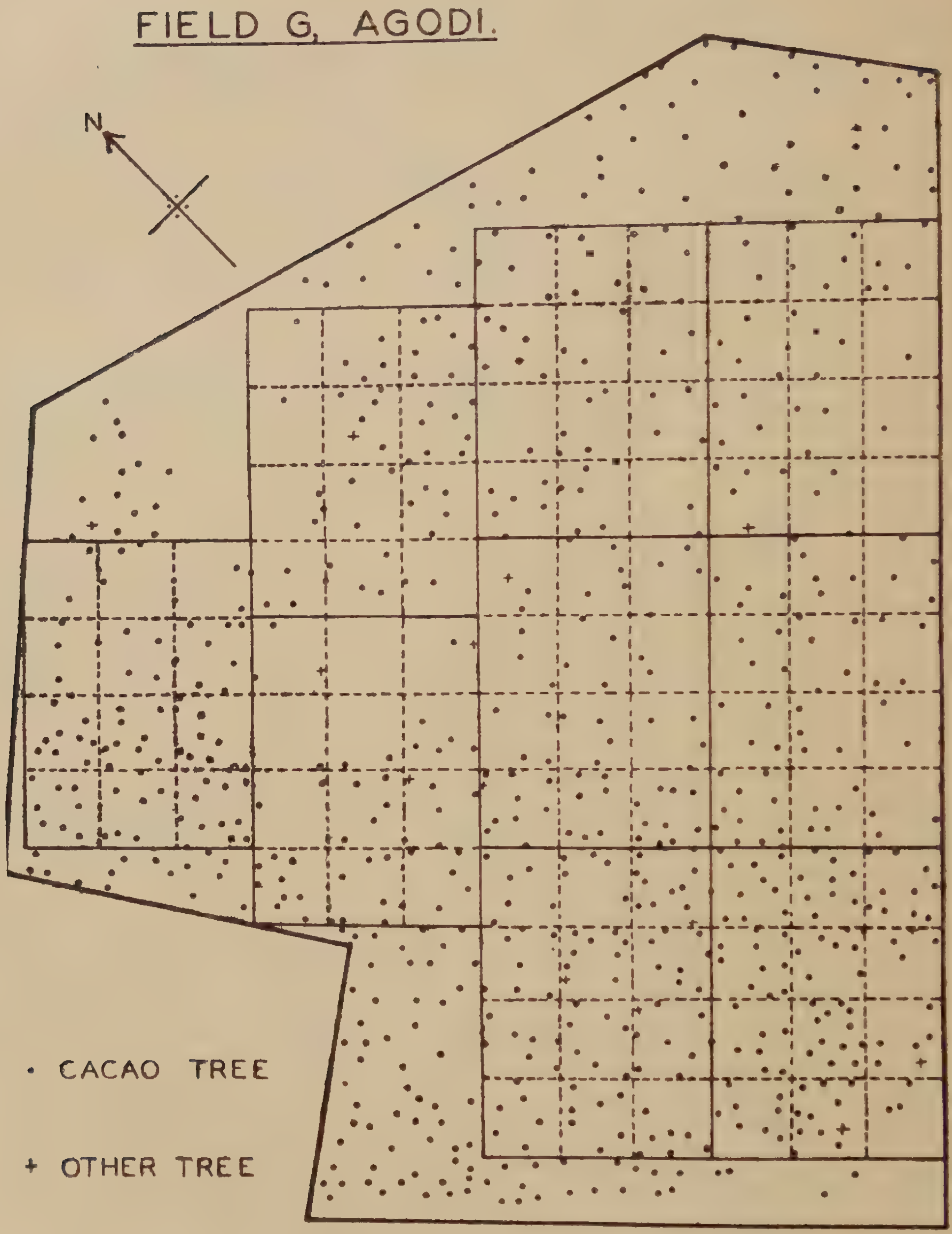
FIELD G, AGODI.
N
• CACAO TREE
+ OTHER TREE

FIG. 1.

Plan of Field G, showing location of cacao and shade trees. The side of a square represents 16·5 feet.

of (i) determining the area occupied by the roots of each tree and (ii) interpreting results on the basis of yield per acre when the desirable planting distance is unknown.

Since economy of land was of paramount importance it followed [7] that attention must be given to small plots and it was decided to work with square units of $\frac{1}{160}$ acre, grouping them in various ways. The high variability to be expected from small plots made it all the more desirable to consider possible means of minimizing error, such as the adding of results from several successive years and their adjustment by covariance upon earlier yields and other measurements.

In an actual experiment it would be necessary to discard strips between the plots, but no account was taken of this in the investigation because it would have meant very much more work, while the introduction of narrow discards would have had little effect upon the conclusions reached.

Trees that lay on boundaries of unit plots were ignored. In an actual experiment it would be better to remove them.

The variate was the number of pods per tree, this being the only one available. It had been chosen by previous investigators, Cheesman and Pound [1] setting out reasons for its adoption. Crops in two-year periods were used so as to eliminate biennial effects, namely, 1943-44, 1945-46, and 1947-48.

The available fields were divided into three classes according to tree density. From the class with most trees per acre, Field J was chosen as being the largest, while similarly G was chosen from the middle class. Naturally, in view of the tree density, a few of the unit plots in G contained no trees. No field was chosen from those with sparse trees because so many of the unit plots would have been empty.

In each field the unit plots were considered in sets of twelve, arranged four by three, as in Figs. 1 and 2. Divisions of these sets are shown in Fig. 3.

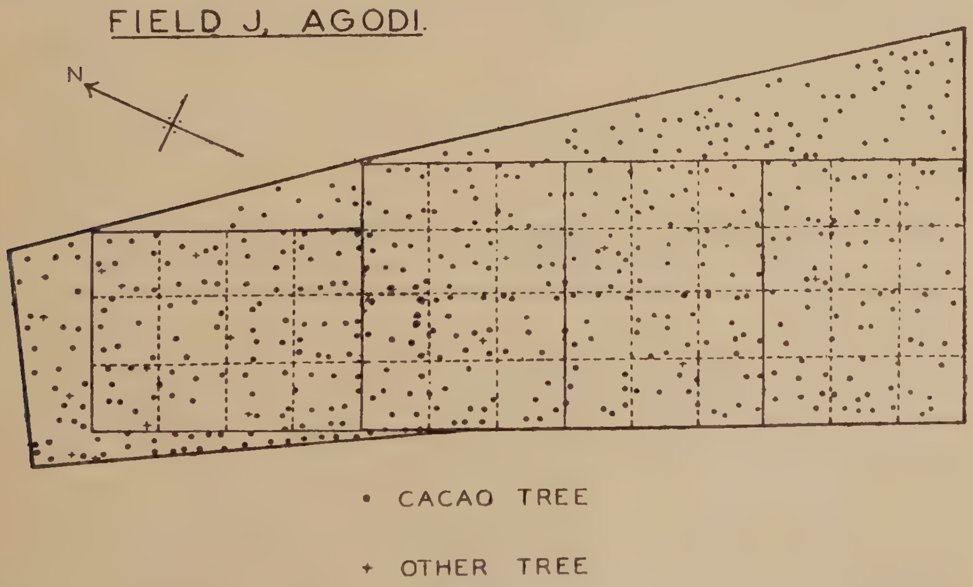

FIG. 2.

Plan of Field J, showing location of cacao and shade trees. The side of a square represents 16·5 feet.

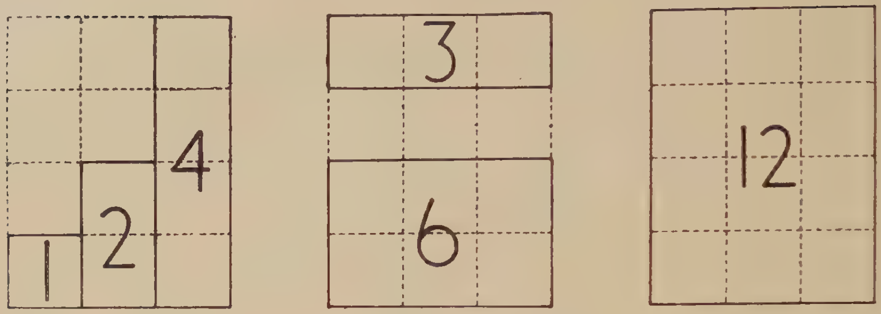

FIG. 3.

Diagram showing the grouping of unit plots into experimental plots and blocks.

In the following work *V* represents the coefficient of variability; while *A* represents the area of land, measured in units of $\frac{1}{160}$ acre, needed for each treatment in order to show differences of 20 per cent. between treatment means. *A* was calculated from *V* using the formula previously given [6]. Also, the notation, 3 : 6, represents plots of three units within blocks of six (Fig. 3) and similarly for other combinations.

Field J being smaller than Field G its values of *V* and *A* are based upon fewer degrees of freedom and are therefore less accurately determined.

RESULTS.

In Table I are set out values for *V* and *A* for crop in a six-year period for various combinations of plot and block-size. Thus in Field G, an experiment in which each block contained two plots each of three units (i.e. two treatments, 3 : 6) it would be necessary to use 31 units of land for each treatment, i.e. ten replicates would be needed.

In both fields, whatever the number of treatments, *V* decreases with increasing plot-size, there being only few exceptions to this. Also, for any given plot-size,

TABLE I.

Values of V and A for different combinations of plot and block-size for crop in a six-year period (1943-48). A is measured in units of $\frac{1}{160}$ acre.

Plot :	Field G.		Field J.		Plot :	Field G.		Field J.	
Block	*V*	*A*	*V*	*A*	Block	*V*	*A*	*V*	*A*
Two treatments.					*Three treatments.*				
1 : 2	44·8	44	38·0	32	1 : 3	50·3	56	32·8	24
2 : 4*	37·2	61	22·2	22	2 : 6	37·2	61	23·6	25
3 : 6	21·6	31	27·4	50	4 : 12	28·6	72	17·4	27
6 : 12	25·1	83	12·5	20					
Four treatments.					*Six treatments.*				
1 : 4*	47·5	50	36·0	29	1 : 6	48·0	51	36·2	29
3 : 12	27·1	49	24·6	40	2 : 12	38·5	65	23·2	24

* In an experiment it would be wise to make these blocks as compact as possible, i.e., two units by two, not four by one. The figures given, therefore, possibly indicate greater variability than would be obtained in an actual trial.

V is not much affected by the number of treatments. Considering next the values *A*, for Field G they tend to increase with increasing plot-size but the results for Field J are inconclusive, probably because of the few degrees of freedom. Similar tables were worked out for crops in the periods, 1943-44, 1945-46, 1947-48, 1943-46 and 1945-48, and all gave similar results, though the general levels of *V* and *A* depended somewhat upon the period considered.

Table II sets out variability in different periods, both with and without covariance corrections. The figures given are for the plot : block combination 1 : 2, but similar tables were prepared for the other combinations as well. The corrections considered were (i) by the number of trees per plot, (ii) by crops in the two years preceding the start of the period and (iii) by other previous crops, the results of these last not being in the body of the table.

TABLE II.

Values of A for a plot of $\frac{1}{160}$ acre within a block of $\frac{1}{80}$ acre for different periods with and without covariance correction. A is measured in units of $\frac{1}{160}$ acre.

Period	Field G.			Field J.		
		Corrected by			Corrected by	
	Uncorrected	Number of trees.	Crop for preceding two years.	Uncorrected	Number of trees.	Crop for preceding two years.
Six years.						
1943-48	44	23	—	32	28	—
Four years.						
1943-46	45	25	—	33	30	—
1945-48	45	23	14	35	32	12
Two years.						
1943-44	52	32	—	34	31	—
1945-46	46	27	16	42	39	18
1947-48	53	26	15	38	33	17

Field G, Crop 1947-48 corrected by crop 1943-46, $A = 15$.
Crop 1947-48 corrected by crop 1943-44, $A = 20$.

Field J, Crop 1947-48 corrected by crop 1943-46, $A = 13$.
Crop 1947-48 corrected by crop 1943-44, $A = 15$.

For the uncorrected figures there is little advantage in taking an experimental period of more than two years. Correction by the number of trees was advantageous, especially for Field G in which the trees were irregularly spaced. Here again there was not much to be gained in uniformity by lengthening the experimental period. Correction by crop in the two preceding years was very advantageous ; but, where it could be studied, correction by a preceding four-year period was for J even better but not for G. Correction of crop in 1947-48 by 1943-44 was also valuable.

The other tables of the series, not here presented, in general led to the same results. Certain exceptions were noted :

(i) Correction by number of trees was of no value with large plots on Field J. A similar tendency was observed in G also.

(ii) Although corrections by the two preceding years' crop were generally very advantageous, on Field J they appeared to be of no value for the combination 6 : 12 for the years 1947-48. Correction by four preceding years was usually better on Field J and often so on Field G.

Otherwise the tables were in very good agreement with Table II.

TABLE III.

Values of V and A for different combinations of plot and block-size for crop in a two-year period (1947-48) corrected by crop in a four-year period (1943-46). A is measured in units of $\frac{1}{160}$ acre.

Plot: Block	Field G. *V*	*A*	Field J. *V*	*A*	Plot: Block	Field G. *V*	*A*	Field J. *V*	*A*
Two treatments.					*Two treatments.*				
1 : 2	25·8	15	24·3	13	1 : 3	25·0	14	21·2	10
2 : 4*	20·4	18	23·0	23	2 : 6	18·3	15	13·4	8
3 : 6	16·6	18	15·6	16	4 : 12	13·8	17	9·0	7
6 : 12	14·5	28	20·7	56					
Four treatments.					*Six treatments.*				
1 : 4*	27·1	16	26·9	16	1 : 6	25·8	15	22·1	11
3 : 12	17·7	21	20·2	27	2 : 12	20·0	18	19·1	16

* In an experiment it would be wise to make these blocks as compact as possible, i.e., two units by two, not four by one. The figures given, therefore, possibly indicate greater variability than would be obtained in actual trial.

Table III is the analogue of Table I for crop in a two-year period corrected by crop in the preceding four years, since this variate has evidently much to commend it. From the figures there presented it appears that $\frac{15}{160}$ to $\frac{25}{160}$ acre per treatment should be enough to show differences of 20 per cent. between treatments. It thus appears that these fields could be used for experimental purposes provided the treatments were not too numerous.

DISCUSSION AND RECOMMENDATIONS.

It is clear from these results that very substantial reductions of error are obtainable by using the analysis of covariance. If successive crops are independent, adding them over a period will effect a reduction of error that cannot be obtained by covariance. If, on the other hand, as here, high or low yields in cacao tend to persist, as observed elsewhere [1, 2, 8], there will be a marked positive correlation between successive crops and covariance will be more effective than simply adding crops over the same period.*

It is evident that more accuracy is obtained by taking crop in a two-year period, corrected by crop in the preceding two years, than by taking crop in the whole four-year period without correction. This might not be practicable if the treatments were of such a kind as to take some years to become effective. The writers suggest that, in the future, experimenters with cacao could advantageously lay down "calibration trials" [5] in which trees are planted as soon as land becomes available and crops are recorded so that an experiment, once decided upon, can be started immediately upon plants of known capabilities. For example, the value of these fields at Agodi as experimental material has plainly been enhanced by the careful records that have now been made for a number of years.

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* Suppose crop in each of two future periods is estimated to be ξ with standard error σ and let the correlation between these two crops be estimated as ρ. Then the coefficient of variability for the sum of the two periods will be $\frac{100\sigma}{\xi}\sqrt{\frac{1+\rho}{2}}$. If, however, the crop in the second period is corrected by covariance upon that in the first the coefficient of variability will be $\frac{100\sigma}{\xi}\sqrt{1-\rho^2}$. The first of these quantities will be the smaller if $-1<\rho<\frac{1}{2}$: the second will be the smaller if $\frac{1}{2}<\rho\leqslant 1$.

When these corrections by covariance can be made, even small plots are unobjectionable on the score of variability. In fact, for economy of land, small plots sufficiently replicated are to be preferred to large ones. There are, of course, a number of important practical reasons for avoiding a large number of very small plots, but an experimenter should be ready to do all that he reasonably can to overcome them.

Since the number of plots to a block appears to have little effect upon variability, the number of treatments possible will be approximately proportionate to the total acreage of land available.

It has already been suggested that $\frac{25}{160}$ acre (or about 0·15 acre) per treatment should be ample, provided small plots (say, of 0·01 to 0·025 acre) are used and the necessary minimum of two years crop records are available for correction of those to come. This means that a field of 0·5 acre or more could effectively be used for a simple field trial of three treatments provided the trees were so spaced that empty plots were infrequent.

SUMMARY.

Optimum plot-size for experiments on Nigerian estate cacao was investigated for two fields at Agodi, the data representing numbers of pods in two-year periods. If economy in land is important, plots should be as small as practicable and corrections by covariance should be made upon crops of the preceding two or four years to reduce error. In this way, if plots are not more than 0·025 acre in area, about 0·15 acre per treatment is needed for a reasonably accurate experiment.

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(*Received* 18/7/50.)

" DIE-BACK " IN RASPBERRIES—A BORON DEFICIENCY AILMENT

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INTRODUCTION.

FOR several years previous to the 1948-49 season, raspberry growers in the Nelson District had been increasingly concerned at the failure of the fruiting canes to develop normally in the spring. In the spring of 1948 death of buds on the canes and failure to produce normal laterals were very marked, giving the bushes the appearance of suffering from a " die-back ". Not only was production of berries seriously reduced but death of the bushes often resulted. In some cases, too, unsatisfactory growth of the bushes appeared to be associated with a generous liming programme which had been carried out for a number of years, but severe losses of bushes occurred even in the absence of lime treatment. There was also a positive correlation between age of the garden and severity of the disease symptoms. Both the most commonly grown variety Red Antwerp and the lesser-grown variety Lloyd George were concerned.

It so happened that on one seriously affected area the Cawthron Institute had for several years been carrying out fertilizer trials, included in which were plots which received supplementary soil applications of borax. In previous seasons the bushes on these borax-treated plots had shown a slight improvement in growth compared with those receiving the usual mixed fertilizer or blood and bone, but the response to borax was not outstanding. However, in the spring of 1948 there was a remarkable difference between the normally developed bushes where borax had been used and the bare canes of bushes receiving ordinary fertilizers only. This is shown in Fig. 1 where the treated bushes in the background are well supplied with laterals and foliage as compared with the bushes of a no-borax plot in the foreground. This photograph also illustrates the way in which the new season's growth comes away in the spring even on badly affected bushes, so that later in the season the bare canes are hidden and the bushes appear relatively normal.

The suggestion from the available information therefore was that this raspberry ailment was due to a deficiency of boron in the soil of affected gardens.

Boron and Raspberries.

Beyond a reference to boron deficiency symptoms in the black raspberry in Maryland (1), where it was reported that a rosetting occurred similar to that accompanying green mosaic, inquiry into the relationship of boron to growth of raspberries seems to have been confined mainly to British Columbia, Canada. Thus in 1937 (2) the Lloyd George variety, following unsatisfactory growth in the Okanagan Valley, was grown in sand culture at the Dominion Experimental Farm, Summerland, B.C., and responded well to added boron. Leaves from the no-boron plants contained only 6·3 p.p.m. of boron in the dry matter compared with 106 p.p.m. in the boron-treated plants. It has also been reported (2) that a die-back condition in raspberry canes was overcome by applying boric acid at 15 lb. per acre. The symptoms were : falling-off in yield, much bud injury and die-back on the ends of the canes. In the year following treatment the canes were taller and with good lateral growth but where no boric acid was used, they were shorter and with bud injury and die-back. Further, McLarty and Fitzpatrick (3) stated that with raspberries no pathological symptoms had been observed in the field although use of boric acid in the gardens gave better growth and increased yields of fruit. In the Fraser River Valley "decline" of raspberries was associated with a low content of boron (4). Atkinson and Wright (5) have reported that in British Columbia all soils on which raspberry growth was poor showed less than 0·20 p.p.m. of water-soluble boron, while all good areas had more than 0·25 p.p.m. The best area showed 0·37 p.p.m. However, no symptoms related to the poor growth or analyses of plant material are given. Harris (6), in an investigation into the nutrition of the red Cuthbert raspberry, found that in sand cultures the complete nutrient solution with or without boron gave the best results. He notes that "what appeared to be some form of mosaic ran through all the series" with the exception of those treatments just mentioned. This observation is of interest since the distortion of the leaves in boron-deficient raspberries in the Nelson District might easily be mistaken for a "mosaic" of virus origin. Harris further states that in field plots, the addition of boron to the fertilizers employed resulted in increased yields of fruit, which were accepted as being also indicatory of growth responses. Liming at the rate of 2,000 lb. of hydrated lime per acre was detrimental. No analyses for boron content of the plants are reported in this paper, nor was lack of boron considered by Harris as a possible factor in this "decline" of the Cuthbert raspberry.

Harris (7) also used boron on the Cuthbert variety at varying rates and found that there were insignificant effects on yield and total carbohydrates of the fruit, but that the vitamin-C and dry matter contents of the berries were significantly increased. He suggested that, although visual symptoms of deficiency were not present, continued use of the soil in question would probably result in symptoms of boron deficiency later becoming apparent.

Berger in a recent review (8) of boron in relation to plant growth has suggested that optimum growth of raspberries may occur at less than 0·1 p.p.m. of available boron in the soil.

Symptoms of "die-back" in Nelson gardens.

The first indication in the spring is a marked delay in the buds coming into leaf or even a complete failure of the buds to burst. A large proportion of the buds may die during the dormant period and in severely affected gardens this may be so complete as to result in bare canes when lateral growth should be taking place in the spring (Fig. 2). Buds which only partly develop show distorted leaves with unusually large petioles and a border of necrotic tissue ("scorch"). This is not seen on leaves grown later in the season. In some cases no further growth than that shown in Fig. 3 occurs. If a dead or weak bud is cut through, including the cane to which it is attached, a brown ring is seen in the pith of the cane. A further stage of growth in a less severely affected bud is seen in Fig. 4. Leaves are small, thin and deeply indented, the result being that a "feathery" effect is produced. The leaves are elongated and run out to long thin points. The deeper and more frequent indentation contributes to this "feathery" appearance. This symptom is very characteristic and persists throughout the season. Less severely affected canes may develop relatively good laterals; commonly the leaves have a crinkled and uneven surface, somewhat suggestive of a virus infection. This is seen on later growth right through the season, even when no " feathery " leaves develop. Such leaves may be found on canes carrying imperfectly developed bud growth (Fig. 5).

Failure of growth on the old canes often produces a premature and rapid growth of new canes from the bases of the old ones which in a favourable season may give a fair crop of fruit. Thus the garden will show a mass of new growth about the old bare canes (Fig. 6). The leaf symptoms already described, other than a few " feathery " leaves, are rarely seen on new canes.

The effective period of boron deficiency seems to be during the first weeks from bud movement. If symptoms do not appear during that period they do not occur later. The effect on growth can be so severe as to cause complete failure of the old canes, when the only fruit harvested that season will come from new cane growth which does not always develop satisfactorily to the harvesting stage. A slight deficiency may not affect the general growth of the bushes or of the fruit and can only be detected by the leaf " feathering ".

Field Survey.

In December, 1948, a survey was made of the incidence of " die-back " throughout the raspberry-growing areas of Nelson. At this time growth was appreciable at the bases of the bushes, but grading was done on the appearance of the old canes which should have been carrying fruiting laterals. Four groups were separated : badly affected, moderately affected, slightly affected, and apparently healthy. In all, 31 gardens were visited ; where two gardens belonging to the same grower were examined, they are marked 1 and 2 against the letter in Table I denoting the garden. Counts were made on the basis of four random groups of 100 bushes each. The percentages of the various grades in the different gardens are set out in Table I. Most of the counts were made in the Motupiko Valley-Tadmor Valley area, this being the most seriously affected section. There was a wide range in the percentages of badly and moderately affected canes ranging from 88 and 89 per cent. in gardens M2 and G to 1 and 7 per cent. in gardens J1 and H1. The soil type in all these gardens is Motupiko sandy loam, derived from the Moutere Hills deposits. In the Orinoco sector the soils are derived from granite ; these were characterized by some very high counts of affected bushes, garden S having all bushes graded as badly or moderately affected.

It is notable, too, that where the grower had two gardens of different ages the incidence of " die-back " was usually appreciably greater in the older garden, especially where the " die-back " was serious. Thus at F1 and F2, where the gardens were 5 years and 7 years old, the totals of the two most affected groups were 37 and 85 per cent., and at M1 and M2, for gardens 4 years and 8 years old, the counts were 20 and 88 per cent. respectively.

A limited number of boron determinations in raspberry leaves showed a relationship between boron content and incidence of die-back. It seemed that 30-35 p.p.m. of boron in the dry matter of leaves on old canes was necessary for healthy development. Available boron, as determined by extraction of the soil with boiling water (9), was higher on those soils where " die-back " was not so serious. The pH values of the soils varied in the top nine inches of soil from 5·0 to 7·4 ; it is on the more alkaline of the Motupiko fine sandy loams that " die-back " was especially bad. This variation in pH value was largely correlated with the liming programmes

TABLE I.

Incidence of " die-back " in raspberry gardens, together with data for boron content of leaves and soil and pH values of the soils.

Garden.	Locality.	Age of garden. Years.	Affected canes : per cent.				Boron in leaves p.p.m.	Available Boron in soil.		pH Value of soil.	
			Bad.	Moderate.	Slight.	Healthy.		0–9 in. p.p.m.	9–18 in. p.p.m.	0–9 in.	9–18 in.
A1	Sherry River	5	22	29	15	34	24·9	0·68	0·63	5·3	5·4
A2	,,	18	25	33	17	25	—	—	—	—	—
B	,,	14	11	19	13	57	44·0	0·60	—	—	—
C	Tadmor Valley	30	6	16	25	53	31·8	—	—	—	—
D1	,,	3	15	33	26	26	—	—	—	—	—
D2	,,	12	27	40	20	13	27·4	—	—	—	—
E	,,	10	19	40	28	13	23·0	0·37	0·34	6·2	5·4
F1	,,	5	11	26	18	45	—	—	—	—	—
F2*	,,	7	37	48	12	3	16·8	0·57	0·53	7·4	7·2
G	,,	4	58	31	11	0	—	—	—	—	—
H1	,,	3	0	7	18	75	—	—	—	—	—
H2	,,	9	15	28	17	40	—	—	—	—	—
J1	,,	6	0	1	4	95	55·3	0·45	0·35	5·0	5·3
J2	,,	11	2	11	11	76	—	—	—	—	—
K1	Glenrae	7	5	20	23	52	—	—	—	—	—
K2*	,,	10	10	32	18	40	22·9	0·37	0·19	5·5	5·2
L	Kiwi	30	14	31	24	31	—	—	—	—	—
M1	Motupiko	4	3	17	27	53	—	—	—	—	—
M2*	,,	8	52	36	7	5	25·2	0·46	0·25	7·2	6·4
N	,,	8	4	26	12	58	28·5	0·28	—	—	—
O	,,	17	7	17	32	44	—	—	—	—	—
P	,,	15	1	8	21	70	33·5	0·28	—	—	—
Q	Orinoco	9	7	20	23	50	28·2	—	—	—	—
R	,,	6	55	37	8	0	—	—	—	—	—
S*	,,	15	88	12	0	0	13·0	0·31	0·36	6·3	6·4
T	,,	35	30	47	19	4	—	—	—	—	—
U	Graham Valley	5	17	28	23	32	—	—	—	—	—
V	Pangatotara	3	12	61	27	0	—	—	—	—	—
W	Brooklyn	?	18	41	23	18	—	0·41	0·40	5·3	5·1
X	Umukuri	3	7	22	33	38	—	1·43	0·99	6·1	5·4
Y	Riwaka	12–15	20	36	24	20	—	—	—	—	—
	General Average		19	28	18	35					

* Experimental Area.

carried on by the different growers. On the present data it is not possible to set a definite figure for available boron which may serve as a standard for health in a Nelson raspberry garden, because the pH value must also be taken into account. On an acid soil of pH about 5·5 or lower an available boron figure of 0·5 p.p.m. appeared to give satisfactory growth but on a neutral soil such a limit was clearly inadequate.

FIELD EXPERIMENTS.

Field trials with borax have been carried out in gardens F2 and M2 situated on the Motupiko fine sandy loam type of soil. Table I shows that both gardens were badly affected with "die-back" in 1948. Chemical data for garden F2 at Rakau are given below. Such data as are available for the Motupiko Valley soils suggest that very similar figures, except for a lower available phosphoric acid content, apply to garden M2 at Motupiko. The Rakau soil, for the 0-9 in. depth, gave the following figures on analysis, expressed as percentages on the dry soil: Base exchange capacity 11·9 m.e.; total exchangeable bases 10·9 m.e.; CaO 9·8 m.e.; MgO 0·8 m.e.; total nitrogen 0·08; loss on ignition 3·8; citric acid-soluble P_2O_5 0·053, and K_2O 0·004. pH 6·7.

The effect of the lime treatment in raising the pH value, the total exchangeable base and the exchangeable lime figures is clearly seen. The soil is well supplied with citric acid-soluble phosphoric acid, but is poorly supplied with available potash.

The mechanical analysis of this soil shows that down to a depth of 18 in. it is a fine sandy loam.

(*a*) *The Rakau Experiment.*

Field Trial. Fertilizer trials using a complete fertilizer, or blood and bone, had been in progress on this area for several seasons before the plots to receive boron were marked out. The rows of bushes of Red Antwerp variety had been annually top-dressed by the owner with blood and bone and superphosphate. Up to 1945 ground limestone had been applied annually at the rate of approximately 1 ton per acre. Two 65-bush rows were each divided into six plots, five of 11 bushes and one of 10 bushes; the following treatments, additional to the standard complete fertilizer, were applied round the base of the bushes:

Row 1	$MgSO_4$	Control	Dolomite	Borax ¼ oz.	Control	Borax ¼ oz.
Row 2	Borax ½ oz.	Control	Dolomite	$MgSO_4$	Control	Borax ½ oz.

There were thus four control plots and each of the other treatments was duplicated. On a basis of 1,200 bushes per acre the two rates of application of borax correspond to 18·75 lb. per acre and 37·5 lb. per acre respectively.

The complete fertilizer consisted of:

4 cwt. Superphosphate (20/22 per cent. soluble P_2O_5);
1½ cwt. Sulphate of potash;
1 cwt. Sulphate of ammonia;
1 cwt. Dried blood (12 per cent. N);

a total of 7½ cwt. per acre. The fertilizers were first applied on 29th September, 1946. In September, 1947, these treatments were repeated but in September, 1948, only the standard complete fertilizer was used. Further applications of dolomite, magnesium sulphate and borax were made in the spring of 1949, the former two substances as before but the borax at half of the previous rates.

Observations made on 24th January, 1947, showed a response, indicated by taller cane growth, to the lower rate of application of borax. There was no response to the higher rate but no toxic symptoms were seen. No response to the magnesium applications was observed. The season was a dry one, especially at harvest time, and doubtless this affected the growth of the canes.

In 1947-48 there was adequate rainfall over the winter, spring and early summer periods with resultant very satisfactory growth of canes. In December, 1947, there was no observable effect from the borax treatments. In the following February some improvement in growth due to the borax treatment was visible. The magnesium treatments had no definite effect on the appearance of the bushes.

The winter and spring of 1948 were unusually dry and by mid-November growth was generally very poor in the garden except for small areas where it was rather better. Soil examinations did not suggest any explanation for the variation. But on the experimental rows it was most obvious that the borax-treated bushes were healthy. The heavier rate of application was a little more effective than the lesser one. The bushes receiving the magnesium applications, especially the dolomite, appeared to be poorer than those of the control plots, which had received the standard complete fertilizer only. It is interesting to note in passing that dolomite has been reported to increase the severity of boron deficiency symptoms in sugar beet to an extent greater than lime (10). The appearance of part of the experimental rows is shown in Fig. 1, with a control (no borax) plot in the foreground and a borax-treated plot on the right of the photograph. Top growth to carry fruiting laterals was satisfactory on the treated bushes but almost absent on the no-borax plot, although young growth was coming away vigorously from the bases of the bushes. By the beginning of December, 1948, the appearance of the garden had greatly improved, but the crop of berries seemed likely to be very poor. Later in the season, in February, 1949, the borax treatments still showed to advantage, with the dolomite-treated plots still very poor but the magnesium sulphate-treated plots slightly better than the controls.

In the early part of the 1949-50 season those bushes which received borax came into leaf earlier and had a much better leaf- and bud-development. The winter and early spring were dry and throughout the season definite benefit from borax was observed. It would seem that, like other crops, raspberries respond more to borax under conditions of low rainfall.

Some plants received borax sprays (0·5 per cent.), but the response, while visible, was not nearly so striking as the effect of soil applications.

Analytical Results. Chemical data were obtained from soil, leaf and fruit samples from the above experimental area. A total of ⅝ oz. and 1¼ oz. of borax per bush had been applied before the soil samples reported below were obtained on 21st February, 1950; the cores were taken in the rows at varying distances from close to the bush to mid-way between bushes. Two depths of sampling were employed, namely 0-9 in. and 9-18 in. Water-soluble ("available") boron and pH values were determined.

TABLE II.

Water-soluble boron in parts per million (p.p.m.) and pH values of raspberry soils at Rakau.

Treatment.	Water-soluble Boron (B)		pH Value	
	0–9 in. p.p.m.	9–18 in. p.p.m.	0–9 in.	9–18 in.
No borax	0·57	0·53	7·4	7·2
¼ oz. borax per bush*	0·72	0·59	7·3	7·1
½ oz. „ „ „*	1·05	0·62	7·2	6·7

* Used at half these rates only in 1949.

These data indicate that the successive dressings have permitted penetration of borax to a depth of at least 18 in. The pH values were higher than is probably desirable and reflect the past practice of liming this garden. It may be noted that soil samples taken of the 0-9 in. depth in September, 1949, before that season's treatments were applied in October, showed a water-soluble boron content of 0·48 p.p.m. for the no-borax plot, to 0·52 p.p.m. for the higher rate of application of borax.

Leaf samples have been available for analysis from both the 1948-49 and 1949-50 seasons. Two series were taken in each season, from laterals on old (previous season's) canes and on new canes. The first mature leaf, usually the fourth from the tip of the lateral, was collected. In 1949 both sets were obtained on 8th February. At this time the leaves from the older growth had begun to deteriorate. Table III indicates that the boron content of the leaves on the treated bushes was greatly enhanced over that of the no-borax or magnesium-treated bushes, being increased six-fold in the older leaves and three- or four-fold in the younger ones. The depressing effect of the dolomite on boron content is seen in the younger leaves particularly. Samples of canes obtained in April, 1949, showed 6·2 p.p.m. of boron in those from the no-borax plots and 10·0 p.p.m. for the more heavily borax-treated plots, but on the 13th June, 1950, the contents of boron were 10·0 p.p.m. and

TABLE III.

Boron content of Red Antwerp raspberry leaves.
(Expressed as parts per million of boron on the dry matter basis.)

Treatment.	Old Growth.	New Growth.	Old Growth.	New Growth.
	8.2.49.	8.2.49.	7.12.49.	24.1.50.
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
No borax	20·8	16·8	20·9	22·3
Borax ¼ oz. per bush . .	123·8	52·5	47·8	95·2
Borax ½ oz. per bush . .	124·9	61·4	58·4	144·4
Magnesium sulphate . .	22·3	12·9	—	37·5
Dolomite	22·3	13·4	—	17·3

15·3 p.p.m. respectively. In 1949-50 leaf samples were obtained from laterals on old canes on the 7th December, 1949, and from young canes on the 24th January, 1950. On the older growth the boron content ranged from 20·9 p.p.m. on the control plots to 58·4 p.p.m. where the ½ oz. of borax per bush had been given in previous seasons. The effects of the half-rate dressings given in September, 1949, were more marked on the young canes, the values for these rising to 95·2 p.p.m. and 144·4 p.p.m. for the two rates compared with 22·3 p.p.m. where no borax was used. Doubtless the borax had penetrated sufficiently by this date to permit uptake of boron from the spring dressing. The dolomite application again gave an outstandingly low figure of 17·3 p.p.m. of boron.

Fruit obtained on 24th January, 1950, showed 8·6 p.p.m. and 21·7 p.p.m. of boron for the no-borax and the ½ oz. of borax treatments respectively. No abnormalities have been noted in fruit from boron-deficient canes so that it is not possible to say what is a desirable figure for the berries.

Analyses for mineral and nitrogen contents of leaves collected on 8th February, 1949, are given in Table IV.

The data in Table IV suggest that the use of borax has resulted in an increase in the soluble ash, lime and nitrogen contents, and a decrease in the potash content. There is a marked contrast between the two types of leaves, the older ones being high in soluble ash and lime but lower in potash, nitrogen and phosphoric acid than the younger leaves. Such a distinction is commonly found in samples of plants of differing age.

(*b*) *The Motupiko Experiment.*

Following a report from a grower in the Motupiko Valley that his raspberry garden was in a very unsatisfactory state, a visit was made to it on 22nd April, 1947. The garden was in a very poor condition ; many of the buds had died as they opened. The fruit crop was consequently poor and, when seen on the above date, the tips of the canes were dying. A history of heavy applications of ground limestone was reported. A soil sample taken at this time gave a high pH value. It was considered that minor element deficiency (or deficiencies) might be concerned in the unsatisfactory behaviour of the garden.

Field Trial. On 8th September, 1947, plots consisting of seven bushes of Red Antwerp variety were marked out and the following treatments were applied :

(1) Control (no minor elements given).
(2) Borax ½ oz. per bush.
(3) Copper sulphate (cryst.) 1 oz. per bush.
(4) Zinc sulphate hydrated 1 oz. per bush.
(5) Manganese sulphate ($4H_2O$) 1 oz. per bush.
(6) Borax and copper, each at the above rates.
(7) Zinc and manganese, each at the above rates.
(8) Borax, copper, zinc and manganese, each at the above rates.

Four replications of each treatment were provided, except for borax plus copper and zinc plus manganese which had two replications only. The chemicals were applied in the solid form close around the bases of the bushes, so that cultural operations would not interfere with their placement.

The soil of this garden is classified as Motupiko fine sandy loam.

TABLE IV.

Mineral and nitrogen contents of Red Antwerp raspberry leaves with and without borax treatment.

(*Expressed as percentages on the dry matter basis.*)

Type of Cane.	Treatment.	Soluble Ash. %	Insoluble Ash. %	Lime. CaO %.	Magnesia. MgO %.	Phosphoric Acid. P_2O_5 %.	Potash. K_2O %.	Total Nitrogen. N %.
Old ..	No borax	9·21	0·91	3·55	0·65	0·44	1·44	2·42
	¼ oz. borax	10·00	0·86	4·18	0·75	0·43	1·21	2·37
	½ oz. borax	10·80	0·99	4·40	0·78	0·43	1·32	2·72
New ..	No borax	5·54	0·81	1·54	0·64	0·54	1·59	3·13
	¼ oz. borax	6·02	0·65	1·85	0·58	0·50	1·46	3·46
	½ oz. borax	6·12	0·70	1·81	0·69	0·49	1·43	3·39

In the following 1948-49 season the minor element treatments were applied in spray form when the leaves were well advanced but before the flower buds were fully developed. Except with borax used alone, freshly hydrated lime was added to a solution of the metallic salt. Borax was used at the rate of 5 lb. per 100 gallons of water; the metallic sulphates were employed at 10 lb. plus 5 lb. of hydrated lime per 100 gallons of water. In the last three treatments that had involved combinations of elements in the preceding year no applications were made. The spray treatments to the other plots were applied on the 15th November, 1948, using sufficient to drench the bushes.

In the 1949-50 season, spray treatments were applied on the 2nd November, 1949, at the same rates as for the preceding season. On this occasion, however, those plots that had originally received compound soil treatments of minor elements now received the same combinations applied as sprays with the exception of treatment 8 (the complete combination) from which zinc was omitted. The quantities used were the same as for the single element treatments. It is possible that where borax was included in the combination spray, some boron may have been precipitated by the excess of lime over that required to react with the metallic salt, but the effectiveness of the borax does not seem to have been appreciably affected by any such precipitation reaction.

The treatments over the three seasons may therefore be set out in the following tabular form:

Season :—		1947-48.	1948-49.	1949-50.
Method of Application :—		Top-dressed.	Spray.	Spray.
Treatments :—	(1)	Control	Control	Control
	(2)	B only	B only	B only
	(3)	Cu only	Cu only	Cu only
	(4)	Zn only	Zn only	Zn only
	(5)	Mn only	Mn only	Mn only
	(6)	B + Cu	—	B + Cu
	(7)	Zn + Mn	—	Zn + Mn
	(8)	B + Cu + Zn + Mn	—	B + Cu + Mn

Field Notes. On the 18th December, 1947, there was a remarkable improvement in the garden compared with its appearance in the previous season. A large part of this was doubtless due to the satisfactory moisture conditions following the winter, spring and early summer rainfall. Even the plots without any borax treatment showed quite good growth. A beneficial effect from the use of borax was to be seen but, by mid-February 1948, this was no longer evident. The rows receiving the complete minor element mixture were not, however, satisfactory, probably owing to the action of the zinc sulphate, because this salt, even when used alone, appeared in the amounts used, to depress the growth of the plants.

In the following season, on the 15th November, 1948, markedly favourable responses to the treatments applied in September, 1947, containing boron, either alone or with other elements, and even with the complete mixture, were seen. On the control (no minor elements) plots the bushes were in poor condition. Later in the season, in February, 1949, the control plots still lacked growth and looked generally unhealthy. While all boron-treated plots were better than those without boron the differences between treatments were not so marked as in the early part of the season. Boron plus copper was particularly satisfactory, even though no further treatment was given in the spring of the 1948-49 season, indicating a carry-over of the effects of the 1947 soil dressings.

Similar relative conditions of the plots were noted in the 1949-50 season, with much more distinct benefit showing from the use of borax in October and November, 1949, than later on in the season. The benefit from the use of borax is clearly seen from Fig. 7, where no-borax and borax-treated bushes are illustrated.

Analytical Results. A soil sample from this Motupiko experimental area, taken in September, 1949, showed a water-soluble boron content of 0·21 p.p.m. for the 0-9 in. depth. Further samples obtained on 21st February, 1950, gave the following figures on the dry soil :

TABLE V.

Boron content and pH value of soil at Motupiko, February, 1950.

Treatment.	Water-soluble Boron.		pH Value.	
	0–9 in. p.p.m.	9–18 in. p.p.m.	0–9 in.	9–18 in.
No borax	0·46	0·25	7·2	6·4
Borax	0·63	0·62	6·9	6·6

The available boron content has been appreciably increased even in the lower levels of soil ; the borax has thus been dispersed into the root zones of the plants. The pH values reflect the past use of lime in generous amounts, and are probably unnecessarily high for raspberries.

Leaf samples were taken on the 8th February, 1949, on the same basis from old and new canes as was described above in the Rakau experiment. Further samples for the 1949-50 season were obtained on the 31st October, 1949, and on the 23rd January, 1950. The boron contents, in parts per million in the dry matter, are set out in Table VI.

In these sets of samples the boron content of leaves from the no-borax plots has remained fairly constant, whether the leaves were derived from old or new cane

TABLE VI

Boron content of Red Antwerp raspberry leaves at Motupiko.

(*Expressed as parts per million on the dry matter basis.*)

Treatment.	Old canes.	New canes.	Old canes.	New canes.
	8.2.49. p.p.m.	8.2.49. p.p.m.	31.10.49. p.p.m.	23.1.50. p.p.m.
No borax	27·9	25·2	24·9	27·0
Borax	197·8	79·3	60·3	196·5
Borax plus copper	137·6	50·6	55·6	146·9
Borax plus copper, manganese and zinc	123·7	58·8	52·4	128·6

growth. The samples from old cane growth obtained in February, 1949, reflect very plainly the effect of the boron applications, the leaves from old canes giving the high figure of 197·8 p.p.m. of boron. No toxic effects were seen, even at this high level of boron. Borax in combination with the other elements has also effectively raised the boron content. On the treated plots, the levels of boron in the leaves from new canes were not so high as in leaves from the old canes, but the effect of the borax is very evident. In 1949-50 the leaves from new canes show the greatest boron content. It will be noted that the " old canes " of October, 1949, were the " new canes " of February, 1949. Moreover the canes were not sprayed until November, 1949, that is, after the first samples of the 1949-50 season were obtained. The data also suggest that copper reduces the uptake of boron.

Samples of new canes of the 1949-50 growth cycle obtained on the 13th June, 1950, contained the following amounts of boron in the dry matter: control (no borax), 9·7 p.p.m.; borax, 23·9 p.p.m.; borax plus copper, 20·8 p.p.m. The lower boron content shown for the last treatment is in line with the results obtained with the leaf samples.

Mineral analyses of leaves from these plots are given in Table VII. In the leaves from the old canes, treatments containing borax have increased the soluble ash, lime, and magnesia contents but have decreased the potash and total nitrogen figures. In the dry matter, the first three constituents are at a somewhat higher level than in the samples from Rakau; on the other hand, potash is lower at Motupiko. With the leaves from the new canes, there are no consistent differences in the estimated concentrations of the several constituents between bushes which had no boron and those which had borax alone or in combination with other elements. The trends of differences in chemical composition of the samples, according as they were derived from leaves from old or young canes, are the same at Motupiko as at Rakau.

Fruit samples gathered on 17th January, 1949, have also been analysed. Their boron, mineral, sugars, nitrogen, and dry matter contents, and pH values, are set out in Table VIII. It is to be noted that the sugar and dry matter values are on the fresh weight basis, whereas the other constituents are on the dry weight basis; pH values were determined on the pulped fruit.

The boron content of the fruit from the no-borax plots was 13·4 p.p.m. Reliable data for the boron content of raspberries have not been found in the literature available so that it is not possible to say what the normal figure should be. Use of borax alone, or in combination with copper and with copper, manganese and zinc, has doubled the boron content under the conditions of the present experiment. As with the leaves, borax has depressed the potash content, while the addition of copper to the borax has apparently reduced the phosphoric acid content. All borax treatments have raised the percentages of dry matter and sugars in the berries. Addition of copper has reduced the effect of boron in increasing the dry matter content. No effect on the pH values of the berries has been seen. The above data may be compared with the report of Harris (7) on the effect of boron and copper, manganese and zinc on certain constituents of the Cuthbert red raspberry. Boron increased the total carbohydrate, although barely significantly, while copper and zinc had no effect; manganese raised the level significantly. All the elements, except copper, increased the dry weight significantly. The behaviour of the Red Antwerp variety is therefore qualitatively the same as that of the Cuthbert variety

TABLE VII.

Mineral and nitrogen contents of Red Antwerp raspberry leaves at Motupiko.
(Expressed as percentages on the dry matter basis.)

Type of Cane.	Treatment.	Soluble Ash. %	Insoluble Ash. %	Lime. CaO %.	Magnesia. MgO %.	Phosphoric Acid. P_2O_5 %.	Potash. K_2O %.	Total Nitrogen. N %.
Old ..	No borax	10·84	0·62	4·51	0·96	0·44	1·23	3·00
	Borax	11·59	0·59	5·02	1·06	0·45	0·99	2·61
	Borax + Cu	12·03	0·62	5·25	1·10	0·43	0·87	2·39
	Borax + Cu + Mn + Zn	11·30	0·67	5·08	1·01	0·35	0·85	2·30
New ..	No borax	6·84	0·32	2·36	0·80	0·64	1·49	3·94
	Borax	6·84	0·37	2·20	0·85	0·65	1·37	4·02
	Borax + Cu	6·22	0·42	1·91	0·77	0·65	1·49	3·97
	Borax + Cu + Mn + Zn	7·10	0·27	2·44	0·74	0·54	1·28	4·00

PLATE I

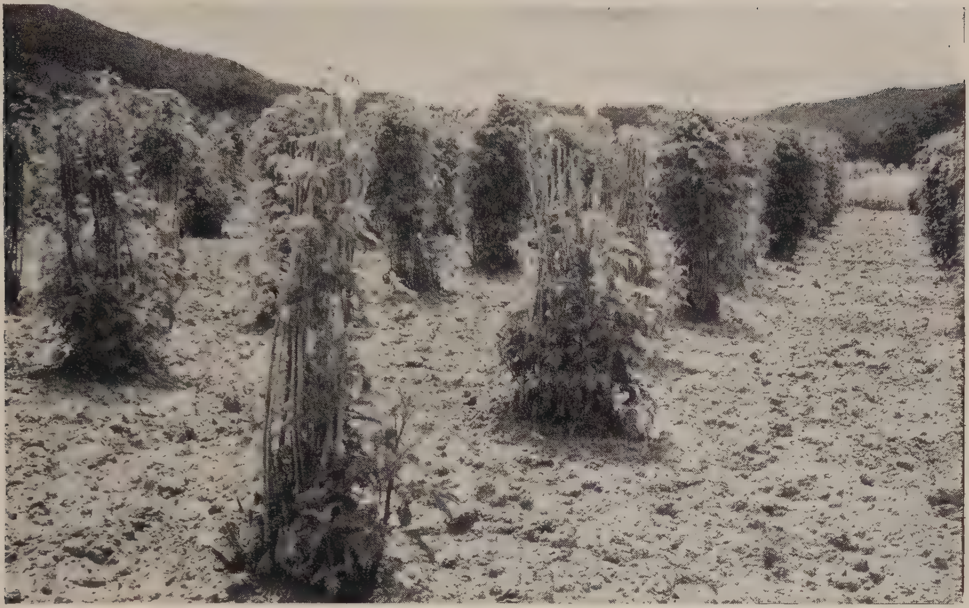

Fig. 1.

Untreated and borax-treated plots at Rakau. Red Antwerp variety.

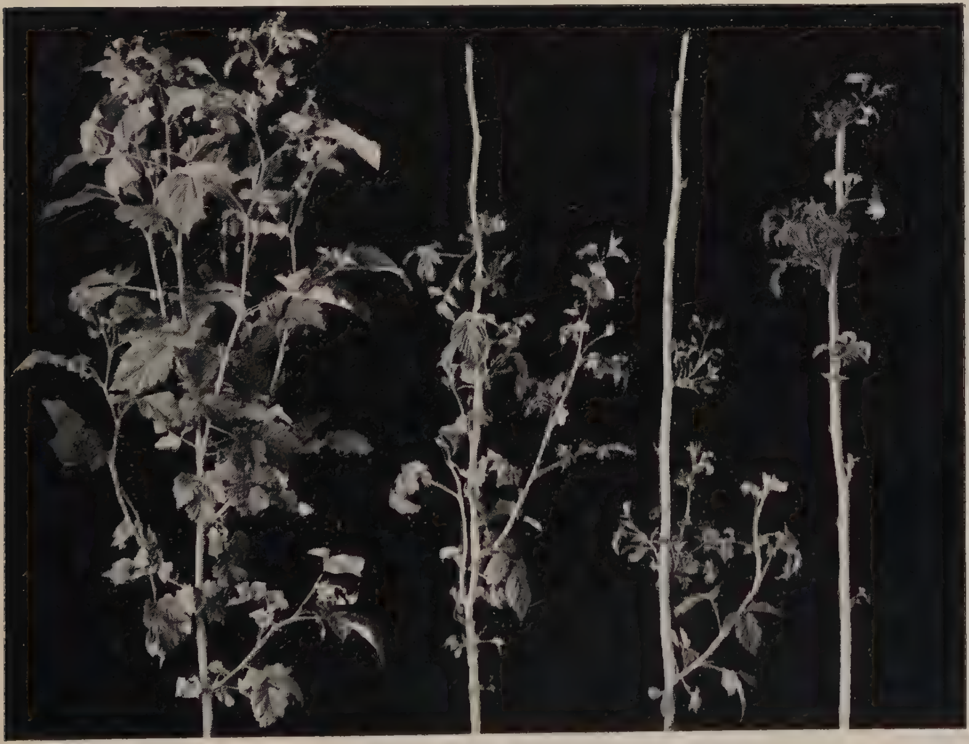

Fig. 2.

Left: Healthy cane.

Others: Canes showing varying degrees of boron deficiency. Red Antwerp variety.

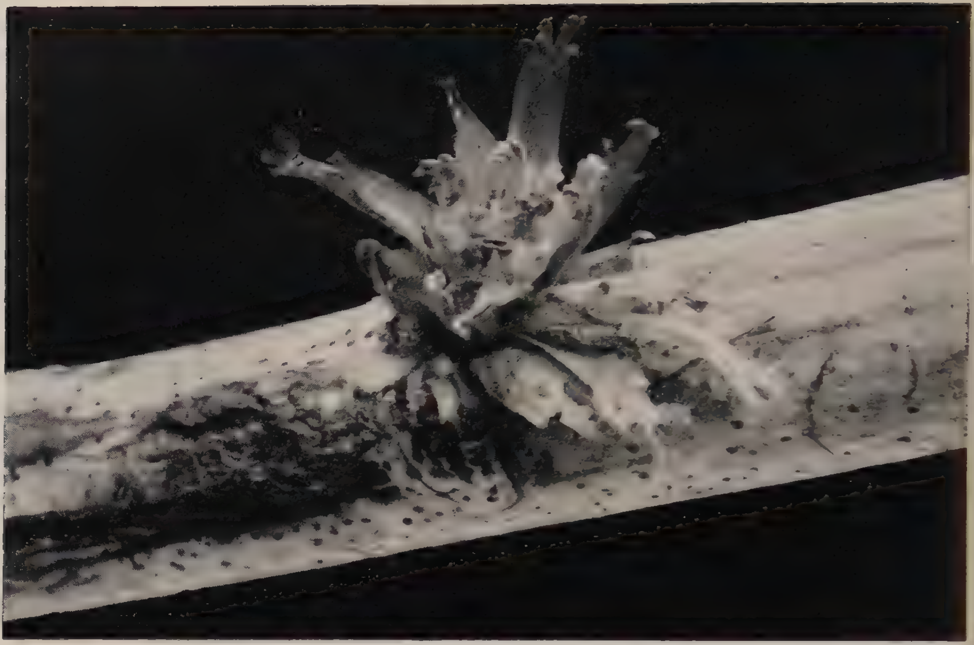

FIG. 3.

Bud with imperfect development and necrosis of edges of leaflets. Red Antwerp variety.

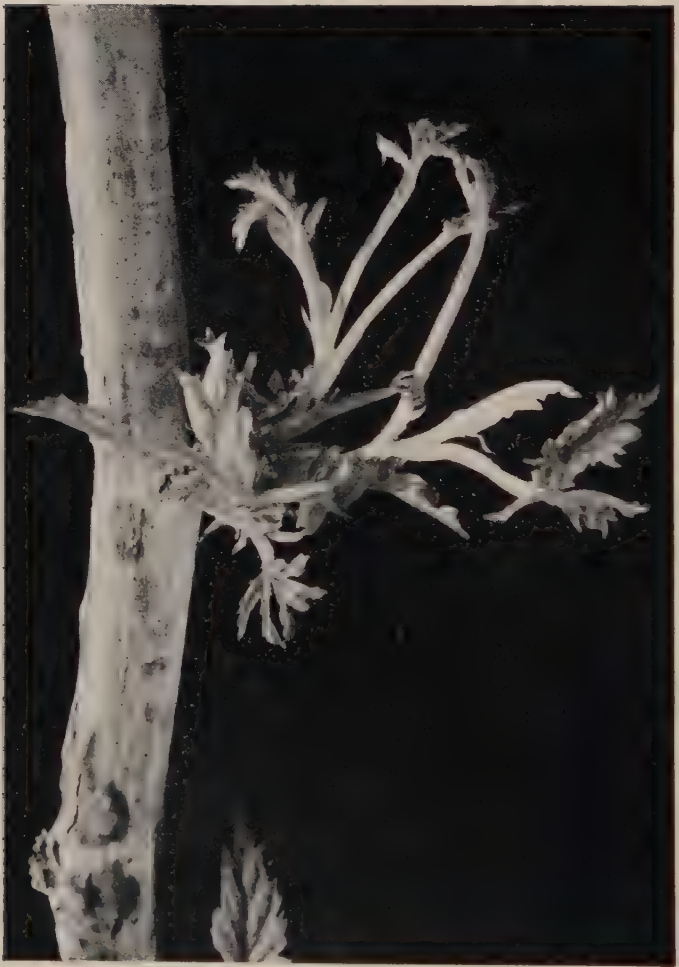

FIG. 4.

Typical growth on boron-deficient cane. The edges of the leaflets are necrotic. Red Antwerp variety.

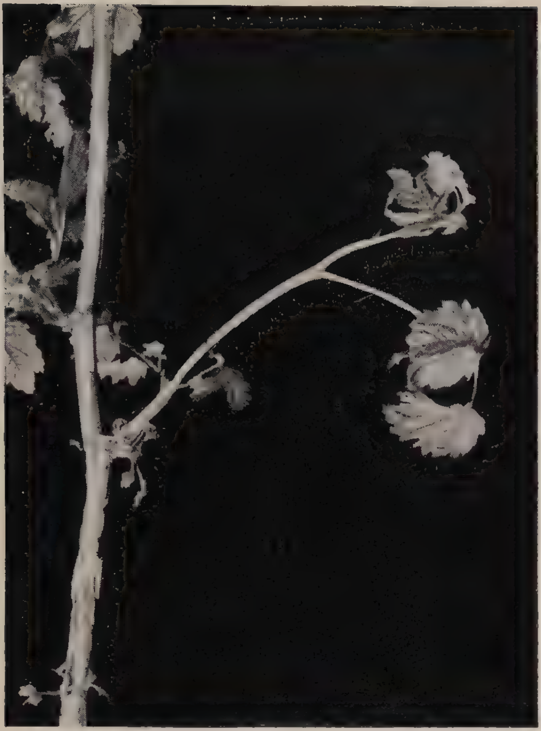

FIG. 5.

Imperfect development of one lateral with crinkled leaves on the other lateral. Red Antwerp variety.

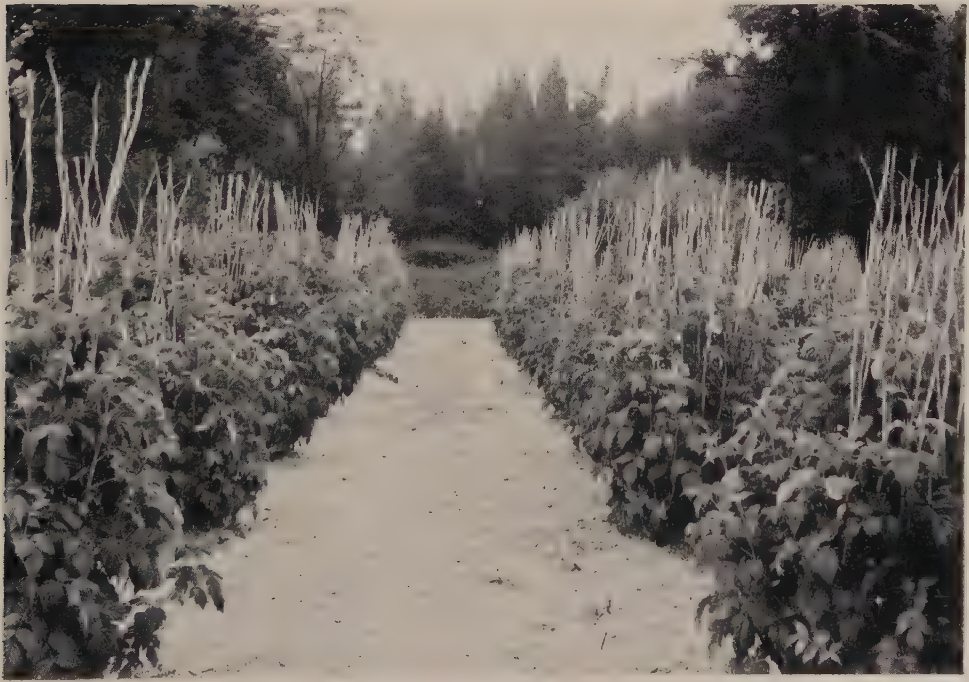

FIG. 6.

Severely affected garden at Orinoco showing profuse basal growth but old canes remaining bare of laterals. Red Antwerp variety.

PLATE IV

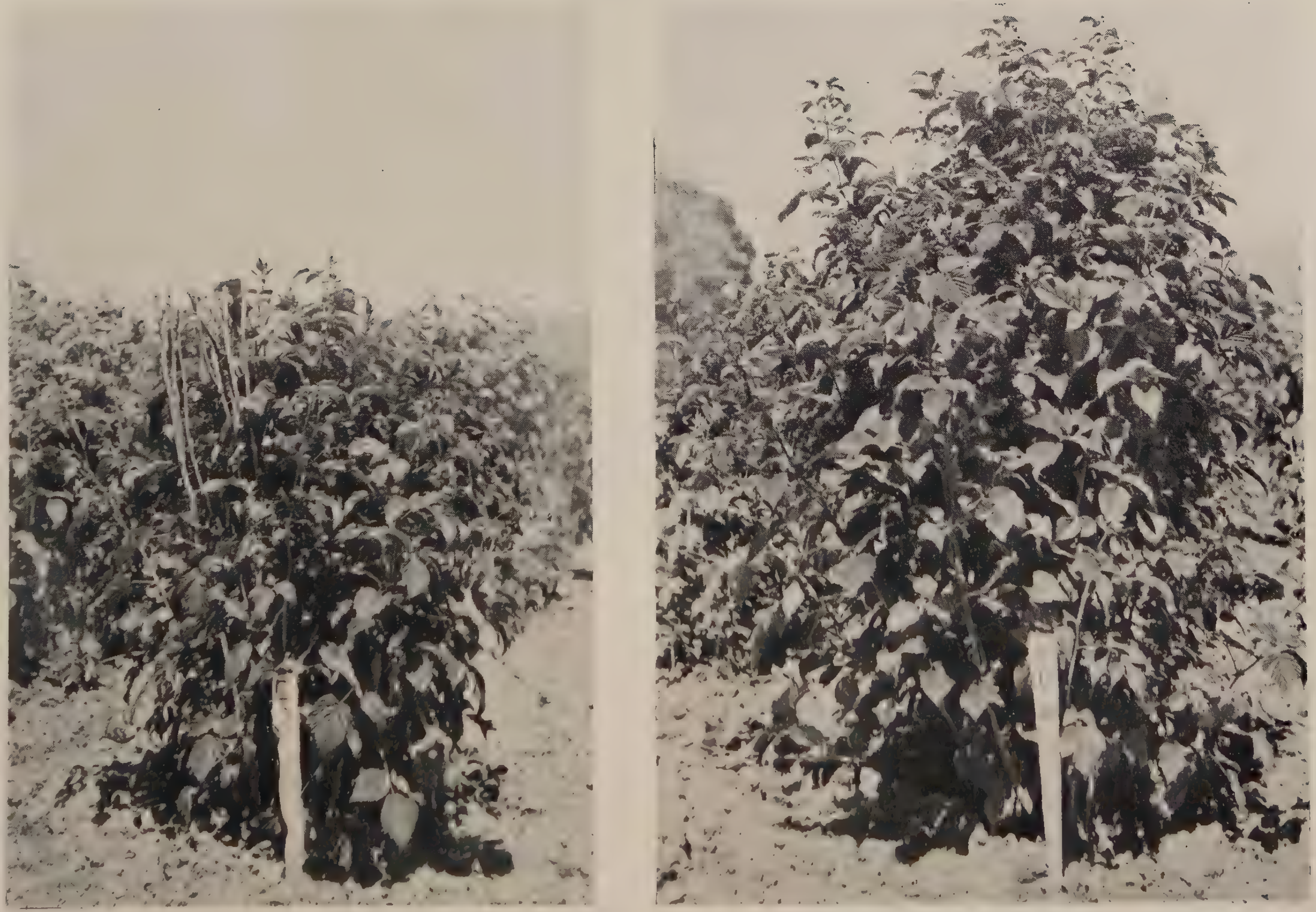

FIG. 7.

Boron-deficient bush on left and boron-treated bush on right, Motupiko. Red Antwerp variety.

TABLE VIII.

Chemical composition of Red Antwerp raspberry fruits from Motupiko.

Treatment.	Boron B p.p.m.	Dry matter.* %	Soluble ash. %	Insoluble ash. %	Lime CaO. %	Magnesia MgO. %	Phosphoric acid. P_2O_5 %	Potash. K_2O. %	Reducing sugars.* %	Sucrose* %	pH value.*
No borax	13·4	12·21	3·07	0·08	0·40	0·29	0·60	1·29	4·23	0·09	2·95
Borax	29·4	14·05	3·00	0·09	0·38	0·25	0·59	1·21	4·84	nil	2·95
Borax + Cu	27·3	13·33	3·18	0·47	0·37	0·30	0·53	1·23	4·95	nil	2·90
Borax + Cu + Mn + Zn	25·3	12·97	2·63	0·19	0·37	0·29	0·49	1·25	4·77	0·22	2·95

* On fresh fruit. All other data on dry matter.

in its response to treatment with these minor elements. It should be pointed out, however, that if Harris' data and the present figures for sugars are converted to the dry matter basis, then the boron effect disappears ; that is, the sugars now are not appreciably different for the several treatments.

(*c*) *Growers' gardens.*

Following a demonstration to raspberry growers in December, 1948, on the experimental areas at Rakau and Motupiko, growers were recommended to treat their gardens with borax at the rate of ½ oz. borax per bush. It was suggested that, while for experimental purposes borax had been placed in a narrow band round the full circumference of the bushes, growers might find it more convenient to apply the requisite amount of borax, corresponding to approximately 40 lb. per acre, mixed with a diluting agent such as sand or dry earth to give a total weight of about one hundredweight, in narrow bands running the length of their rows and placed close to the bases of the bushes. Several growers subsequently did top-dress at any rate parts of their gardens, and samples of soil and leaves from a number of treated gardens have been obtained. The precise method of applying the borax and amounts used cannot be stated for each individual case, but the data of Table IX leave no doubt as to the effectiveness of the applications in raising the level of the boron in the soil and in the dry matter of the leaves.

Three sets of leaf samples were available from certain gardens, those of the 8th February, 1949, being obtained from new cane growth two months after the demonstration already referred to. In the short time available for the borax to be

TABLE IX.

Boron content of soil and of Red Antwerp raspberry leaves following use of Borax.

Garden.	Treatment.	Boron (B) in leaves.			Water-soluble Boron (B) in p.p.m. in soil.	
		8.2.49. p.p.m.	25.11.49. p.p.m.	24.1.50. p.p.m.	0–9 in.	9–18 in.
A	No borax ..	24·9	26·0	60·4	0·68	0·63
"	Borax ..	51·9	70·0	110·5	1·01	1·00
J	No borax ..	55·3	41·6	38·2	0·45	0·35
"	Borax ..	153·6	65·1	164·6	0·68	0·61
E	No borax ..	—	23·0	37·4	0·37	0·34
"	Borax ..	—	104·0	221·9	0·57	0·45
K2	No borax ..	22·9*	40·6	81·0	0·37	0·19
"	Borax ..	—	85·2	127·2	0·71	0·45
S	No borax ..	13·0	—	21·5	0·31	0·36
"	Borax (40 lb.)	—	—	38·4	0·34	0·35
W	No borax* ..	—	—	—	0·41	0·40
"	Borax ..	—	40·1	76·7	0·91	0·59
X	Borax (56 lb.)	—	51·6	131·9	0·60	0·36
D	No borax ..	—	27·4	56·5	—	—
"	Borax ..	—	84·1	189·3	—	—
G	Borax ..	—	143·4	130·3	—	—
B	No borax ..	44·0*	38·6	—	—	—
C	No borax ..	—	31·8	50·1	—	—
N	No borax ..	28·5*	24·5	56·8	—	—
P	No borax ..	33·5*	23·1	49·6	—	—

* Sampled on 4.4.49.

washed down into the root zone, absorption of boron by the plants has been appreciable. In the following 1949-50 season, very marked increases in boron content have been found in leaves from both old and new canes. It is apparent, too, that penetration of borax through the top 18 inches of soil has been satisfactory. An interesting case is that of garden S where the response to boron treatment has been slight. This garden is situated on a very sandy soil so that drainage is probably excessive with the result that the borax is leached out very rapidly. The samples from gardens B, C, N and P, where borax was not used, show the type of variation occurring in raspberry leaves.

DISCUSSION.

The results of field and laboratory work reported in this paper indicate very definitely that one type of unsatisfactory development of raspberries in the Nelson district has been due to lack of available boron in the soil. At the present time it is not possible to state a figure for the level at which available boron should be held for satisfactory growth. For example, deficiency symptoms have been present in gardens E and K2 to a considerable degree, but not to any extent in garden J with only a slightly higher available boron content in the top-soil. The data for gardens J and K suggest that the content of boron in the subsoil may be a determining factor as to whether symptoms will appear or not on two soils of similar available boron content in the top-soil. It is also clear from the field experiments that weather conditions will have a marked influence on the appearance and severity of deficiency symptoms in any given season. The effect of applications of ground limestone must also be taken into account, because there is no doubt that raising the pH value of the soil to approximately 7·0 renders such boron as may be present in the soil less available to the plant. Detrimental effects from liming will be more pronounced on the freer and more sandy soils. For Nelson conditions the available boron in the soil should be at a higher level than the 0·1 p.p.m. suggested by Berger (8) for American conditions.

Analysis for boron content of foliage shows whether the supply of boron is on the low side and is particularly useful in following up the effects of borax applications. Care must be taken, however, to separate leaves from fruiting canes and those from freshly grown ones : the latter are usually appreciably higher in boron content than the former, even in untreated gardens. For leaves from fruiting canes, about 35 p.p.m. of boron on the dry basis should be present for satisfactory growth but for leaves from new canes, a normal figure would appear to be 50-60 p.p.m.

Raspberries seem to tolerate relatively large applications of borax, as the field trials reveal. Even with the use of ½ oz. borax per bush, boron contents of over 300 p.p.m. in the dry matter of leaves from new canes have been observed without any signs of toxicity. On the other hand young plantings may require more circumspection in the use of borax ; for example one such area that received ½ oz. of borax per bush carried many plants showing scorched leaves suggestive of boron toxicity. Analysis of leaves from these plants indicated a boron content of 293 p.p.m., whereas leaves from untreated plants contained only 51·6 p.p.m. in the dry matter. These figures suggest, too, that foliage on young plants may be more sensitive to excessive amounts of boron, because, as noted already, leaves from fully grown bushes can tolerate a higher concentration than 293 p.p.m. without showing any toxic effects.

SUMMARY.

Unsatisfactory growth due to failure of buds to develop on fruiting canes of raspberries has been shown to be due to lack of available boron in the soil of affected gardens. Leaf symptoms are described. The popular name " die-back " has been applied to this condition in the raspberry.

The degree of symptom expression varies with seasonal conditions and the past history of the garden. Die-back is more severe in old-established gardens. The lower the available boron content of the soil and the lower the boron content of the leaves the more severe is the ailment.

Borax at the rate of ½ oz. per bush has proved very satisfactory in the control of die-back. Leaves on fruiting canes and on new canes show widely different boron contents. Increased uptake of boron is shown to modify the mineral composition of leaves and fruit on treated bushes. Other minor elements may affect the uptake of boron.

While established bushes appear to be fairly tolerant to borax, care in the use of this chemical is necessary on newly established plants.

ACKNOWLEDGMENTS.

The authors wish to thank Messrs. E. Christian and H. Brown of Rakau and Mr. G. L. Mead of Motupiko for making their gardens available for experimental work and for carrying out the cultural operations.

They are also indebted to Misses K. E. Currie and J. Watson for assistance with chemical analyses of leaves and fruit, and to Mr. L. Hodgson for some of the soil data. Mr. G. Ward prepared the samples for chemical examination.

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(*Received* 15/9/50.)

SPACING EXPERIMENTS ON VEGETABLES

III. THE GROWTH AND YIELD OF SHALLOTS IN RELATION TO SPACING, MANURING AND SIZE OF PLANTING MATERIAL, IN CHESHIRE, 1948

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INTRODUCTION.

OUR common vegetables all have a long history of cultivation, and present methods of culture derive from traditions which embody the experiences of the past. In the absence of quantitative data, it is not possible, however, to state with certainty the effect on yield of altering various cultural practices. Nowhere is this lack of precise information more evident than in the relation between spacing and yield. Thus, with shallots, recommended spacings vary from 9 in. × 4 in. (Min. of Agric., 1948) to 12 in. × 12 in. (Johnstone, 1948), and such variations, apart from any effect on yield, greatly alter the cost of planting a given area to this crop. Possible relationships between spacing and soil fertility or size of planting material remain almost wholly unexplored. Hoare (1945) states that small bulbs give the larger crops and a higher proportion of large daughter bulbs, whilst Stuart (1947) reports that small cloves (bulbs) produce a low yield of large shallots and big cloves give a good yield of small daughter bulbs. Stuart's data, however, show only a slightly (and possibly not significantly) reduced yield from planting small bulbs. Stuart's figures show that with rows 12 in. distant, reducing the planting distance in the row from 9 in. to 4½ in. led to an increase of approximately 40 per cent. in the total yield.

The paucity of information led us to investigate the effects of varying the size of the planting material, the planting distance, and the manurial treatment, on the growth and yield of Dutch yellow shallots. Many of the results obtained are so clear-cut as to merit reporting at this stage.

GENERAL EXPERIMENTAL PROCEDURE.

The experiment was carried out at the University of Manchester Jodrell Bank Experimental Station, Lower Withington, Cheshire, on a heavy loam which has been generously manured for several years and is in a high state of fertility. The site was winter ploughed to a depth of 8 in., limed at the rate of 1¼ tons of hydrated lime per acre in early February, and fertilizers applied (to the manure plots) late in February and harrowed in.

The effects of the following treatments were investigated :

(1) Manures : (a) no manure, and (b) 10 cwt. per acre of a mixture of superphosphate of lime 2 parts, sulphate of ammonia 2 parts, and sulphate of potash 1 part.

(2) Row spacings : 8 in., 12 in. or 16 in.

(3) Plant spacings : 3 in., 6 in., 9 in. or 12 in.

These combinations of row and plant distances gave a range of plant populations from 32,670 to 261,360 per acre.

(4) Size of planting material : The stock of virus-free Dutch yellow shallots as received was riddled on a 1¼ in. riddle to give two sizes of planting material, "large", averaging 20 per lb., and "small", averaging 53 per lb.

The experiment was arranged in three randomized blocks. Each of the combinations of manure and row spacing was repeated in six main plots within each block, these plots being " split " (Snedecor, 1946) for the eight combinations of plant spacing and size of planting material.

The bulbs were planted on 9th and 10th March, 1948. Post-planting interference by birds was slight, but all displaced bulbs were replaced daily until they became established. Counts of plants growing on 16th April showed a mean value for all plots of 98·2 per cent. of the theoretical number with 99·4 per cent. for the " large bulb " and 96·9 per cent. for " small bulb " plots. These deviations from theory are so small that in subsequent calculations it is assumed that the theoretical number of plants was present.

The plants on all the plots grew well, favoured by a forward spring. The weather was extremely dry in May, and certain plots exhibited a marked yellowing of the foliage ; on all plots a record of foliage colour in categories was made on 19th May and 25th May and again on 15th June, after heavy rain.

Bolting was conspicuous on all the " large bulb " plots, but slight on the " small bulb " plots. In spite of continued heavy rain in June and July, the closely spaced plots ripened early, whilst the widely spaced plants continued to split. The plots were harvested on 4th, 5th and 6th August, dried and rubbed out. Data were collected for each plot for (*a*) total number of bulbs ; (*b*) number of " bolters " ; (*c*) total yield ; (*d*) yield of bulbs over 1¼ in. diameter (equal to approximately 0·5 oz.) ; (*e*) yield of bulbs between ¾ in. and 1¼ in. diameter (more than 0·2 oz. and less than 0·5 oz. approximately and (*f*) yield of bulbs of less than ¾ in. diameter. These grades were taken to indicate approximately the yields of bulbs (*d*) large enough to serve as an onion substitute (*e*) of a size suitable for pickling purposes (and as our results show, suitable for planting) and (*f*) of little value.

RESULTS.

(1) *Bolting.*

Bolting was appreciable only on the plots from large bulbs and here its incidence was affected by the spacing but not by manuring. The number of inflorescences per plant for the 8 in., 12 in., and 16 in. rows was 0·69, 0·89 and 1·09, and for the 3 in., 6 in., 9 in. and 12 in. plant spacings, 0·73, 0·90, 0·95 and 0·99.

(2) *Ripening.*

No quantitative expression of ripening was attempted but by 23rd July all the 8 in. × 3 in. large, 8 in. × 3 in. small, 8 in. × 6 in. large, 8 in. × 9 in. large, 12 in. × 3 in. large and 16 in. × 3 in. large plots were ripe, or nearly so, and fit to lift. The plants on the other plots carried varying amounts of green foliage.

(3) *Bulb number.*

Counts at harvest of the total number of daughter bulbs show that for each row spacing the number of daughter bulbs per plant increases with each increase in the planting distance. The values for the 3 in., 6 in., 9 in. and 12 in. spacings were 6·9, 8·5, 8·9 and 9·9 in 8 in. rows, 7·2, 8·4, 9·0 and 9·8 in 12 in. rows, and 8·1, 9·2, 10·0 and 10·1 in 16 in. rows, and these effects persist if " large " and " small " plants are considered separately, although the mean value for the large bulb plants (11·4) is much greater than that for the small bulb plants (6·4). This effect of the size of the planting material is evident at all spacings and manurial levels.

TABLE I

Total yield of shallots (pounds per plot) grown at several spacings and two manurial levels.

Row distance.	Planting material.	Planting distance.								Means.		Manurial treatment.	
		3″		6″		9″		12″				No manure.	Manure.
8 in.	Large	30	$\mathbf{33}^{e}$	33	$\mathbf{32}^{e}$	32	$\mathbf{32}^{e}$	31	$\mathbf{29}^{e}$	31	$\mathbf{31}^{a}$	29^{c}	34^{c}
	Small	36		32		31		27		31			
12 in.	Large	31	$\mathbf{32}^{e}$	30	$\mathbf{30}^{e}$	28	$\mathbf{27}^{e}$	24	$\mathbf{23}^{e}$	28	$\mathbf{28}^{a}$	26^{c}	30^{c}
	Small	34		29		27		23		28			
16 in.	Large	33	$\mathbf{33}^{e}$	27	$\mathbf{26}^{e}$	26	$\mathbf{24}^{e}$	23	$\mathbf{21}^{e}$	27	$\mathbf{26}^{a}$	25^{c}	27^{c}
	Small	33		26		22		20		25			
Means	Large	31^{f}		30^{f}		29^{f}		26^{f}		29	$\mathbf{28 \cdot 5}$		
	Small	34^{f}		29^{f}		27^{f}		23^{f}		28			
Manurial treatment.													
No manure		30^{d}		28^{d}		25^{d}		25^{d}		27			
Manure		36^{d}		31^{d}		29^{d}		24^{d}		30			
Means		33^{b}		30^{b}		27^{b}		24^{b}		28·5			

Treatments exerting a significant effect:

Row distance	(a)	P = <0·01
Plant distance	(b)	P = <0·01
Interaction of:		
Manure and row distance	(c)	P = <0·05
Manure and plant distance	(d)	P = <0·05
Row distance and plant distance	(e)	P = <0·05
Size and plant distance	(f)	P = <0·05

(4) *Total yield.*

Data for the total yield of bulbs in pounds per plot are summarized in Table I. In this, and subsequent Tables, values in black type represent the means of the two values on the left and here as throughout the paper, the term " significant " is applied to a difference which is of such a magnitude that it would occur by chance not more than once in twenty times, but the level of probability is shown against the significant effects in the Tables. These results require only brief comment at this stage. The effect of manure on yield just fails to reach the level of significance, but the interactions, manure × row distance, and manure × plant distance, are significant. Yield falls as the row distance or the plant distance increases from 8 in. to 16 in., or from 3 in. to 12 in. respectively, and the manurial response is greatest at the closest row and plant spacings. The interaction of row spacing × plant spacing is significant, and for each increase in row distance the effect of increasing plant distance in reducing yield becomes more evident. Although size of planting material is without significant effect on the yield, the interaction of size × plant distance is significant, and that for size × row distance nearly so. The result of this is that whilst at close spacings the " small " plants out-yield the " large " ones, the reverse is true or there is no appreciable difference at the wide spacings.

The combined effects of the interactions size × row distance, size × plant distance, and plant distance × row distance are such that maximum yields result only when small bulbs are planted closely and, for these plants, every increase in the spacing over 8 in. × 3 in. causes a decreased yield. For the " large " plants in the 8 in. rows, plant distance is without effect on the yield, but as the row distance increases, the depressing effects on yield of increasing the plant distance become evident. Similarly, for the 3 in. plant spacings increasing the row distance does not decrease the yield, but for all other plant spacings increasing the row distance decreases the yield, and this effect is greatest at the widest plant spacings. For any assessment of profit, allowance must be made for the weights of material planted, and this will differ according to the size of the material planted, and the spacing employed. Table II shows for large and small plants separately the gains at harvest over the weights planted.

The beneficial results which follow close planting of small bulbs become even

TABLE II.

Net Yields (i.e. gains over weight planted) of Shallots.
Pounds per Plot.

	Large bulb plants.					Small bulb plants.				
	Plant distances.					Plant distances.				
Rows.	3″	6″	9″	12″	Means.	3″	6″	9″	12″	Means.
8 in. ..	16·1	25·1	26·5	26·7	23·6	31·0	29·2	28·8	25·9	28·7
12 in. ..	22·1	25·3	24·4	21·9	23·4	30·9	27·0	25·1	21·6	26·2
16 in. ..	25·7	23·7	23·6	17·4	22·6	30·4	24·2	22·4	19·3	24·1
Means ..	21·3	24·7	24·8	22·0		30·8	26·8	25·4	22·3	

more apparent now than when gross yields were considered. It is clear, therefore, that interest centres on these plots and a consideration of the gains in yield due to manuring suggest that the beneficial results of a close planting of small bulbs are most likely to result when nutrient supplies are abundant.

The effects of competition in restricting growth are more readily appreciated if the yield data are transformed into mean weights per plant, and this has been done in Table III.

TABLE III.

Mean weight (in ozs.) of shallot plants grown at several spacings.

	Large bulb plants.					Small bulb plants.				
	Plant distances.					Plant distances.				
Rows.	3″	6″	9″	12″	Means	3″	6″	9″	12″	Means.
8 in. ..	1·7	3·6	5·2	6·8	4·3	2·0	3·5	5·1	6·0	4·2
12 in. ..	2·6	5·0	6·9	8·0	5·6	2·8	4·8	6·6	7·5	5·4
16 in. ..	3·6	6·1	8·6	9·9	7·1	3·7	5·6	7·2	8·8	6·4
Means ..	2·6	4·9	6·9	8·2	5·7	2·8	4·6	6·3	7·4	5·3

The spacing effects in relation to size of planting material are of especial interest. For the large plants in 8 in. rows increases in the plant distance result in proportionate increases in growth. Similarly, for the 3 in. plant distance, increases in the row spacing give a proportionate increase in growth. As the row distance increases, the response of the individual plants to the increased row space available becomes relatively, although not absolutely, less, so that for the 12 in. and 16 in. row distances, increasing the plant distance results in a less effective exploitation of the soil. With the " small " plants, which have a smaller number of daughter bulbs per plant, increasing either the row distance or the plant distance does not at any plant or row spacing employed give a proportionate increase in plant weight, although the increase is almost proportionate for the 3 in. plant distance.

(5) *Mean weight of daughter bulbs.*

Calculated values for mean daughter bulb weight are summarized in Table IV. Manuring increases the bulb weight slightly (from 63 to 69 oz. per 100 bulbs). Daughter bulbs resulting from the planting of small bulbs are larger than those from large bulbs at every spacing. Each increase in plant spacing for every row distance, and each increase in row distance for every plant spacing increases the mean bulb weight for both " large " and " small " plants.

(6) *Yields of several grades of bulbs.*

The total crop for each plot, after discarding bolters, was graded into three fractions, over 1¼ in. diameter (suitable for use as onion substitutes), ¾ in. to 1¼ in. diameter (suitable for pickling and planting) and below ¾ in. diameter (considered as mainly too small to be of value). The yields of the first two of these grades for the

TABLE IV.

Weight (in oz.) per 100 daughter bulbs for shallots grown at several spacings.

Row distance.	Planting material.	Planting distance.				Means.
		3″	6″	9″	12″	
8 in.	Large Small	19 **30** 42	30 **49** 67	52 **62** 72	53 **71** 90	38 **53** 68
12 in.	Large Small	30 **41** 52	48 **64** 81	61 **79** 97	65 **85** 106	50 **67** 84
16 in.	Large Small	35 **52** 69	51 **70** 88	66 **85** 104	76 **103** 130	57 **77** 98
Means	Large Small	28 **42** 55	42 **61** 79	60 **76** 91	64 **87** 109	49 **66** 83
No Manure Manure	Large	25 **28** 30	41 **43** 44	52 **59** 67	67 **65** 61	46 **49** 51
No Manure Manure	Small	51 **54** 57	78 **79** 79	84 **92** 98	105 **108** 112	80 **83** 87
No Manure Manure		38 **41** 44	59 **61** 62	68 **75** 83	86 **87** 87	63 **66** 69

different treatments are shown in Tables V and VI, which are followed by statements of the salient features emerging from an analysis of the data.

As the grades over 1¼ in. diameter and between ¾ in. to 1¼ in. diameter correspond closely with the "large" and "small" planting material used, these terms will be used to describe these two fractions of the total crop. The data which are presented need to be interpreted cautiously, as small alterations of the grading limits or of the mean bulb weight, may greatly alter the proportion of the crop falling into a particular grade.

From Table V it is seen that manuring increases the yield of large bulbs, and this increase is greatest at the close spacings. The yield of large bulbs is greater when small rather than large bulbs are planted at each of the spacings employed, and this increase in the yield of large bulbs consequent upon the planting of small ones, is greatest when the plants are closely spaced. The interaction, row distance × plant distance is significant, so that as the plant spacing increases the effect of altering the row distance decreases. Also, the second order interaction of size × row distance × plant distance is almost significant, and with the plants spaced at 3 in. the effect of increasing the row distance has little effect with large plants, but has a large effect with small plants. The maximum effect of increasing row distance is,

TABLE V.

Yield of large (over 1¼ in. diameter) shallot bulbs (pounds per plot) grown at several spacings and two manurial levels.

Row distance.	Planting material.	Planting distance.				Means.	Manurial treatment.	
		3″	6″	9″	12″		No manure.	Manure.
8 in.	Large	1^{k}	3^{k}	9^{k}	10^{k}	6	8^{e}	13^{e}
		4^{g}	**10^{g}**	**14^{g}**	**14^{g}**	**10^{b}**		
	Small	8^{k}	16^{k}	19^{k}	18^{k}	15		
12 in.	Large	3^{k}	8^{k}	14^{h}	12^{k}	9	10^{e}	15^{e}
		8^{g}	**13^{g}**	**16^{g}**	**14^{g}**	**13^{b}**		
	Small	13^{k}	17^{k}	19^{h}	17^{k}	16		
16 in.	Large	4^{k}	9^{k}	11^{h}	14^{k}	9	12^{e}	14^{e}
		10^{g}	**13^{g}**	**13^{g}**	**14^{g}**	**13^{b}**		
	Small	17^{k}	17^{k}	16^{k}	13^{k}	16		
Means	Large	3^{h}	7^{h}	11^{h}	12^{h}	8^{d}		
	Small	12^{h}	17^{h}	18^{h}	16^{h}	16^{d}		
Manurial treatment.								
No manure		5^{f}	10^{f}	12^{f}	14^{f}	10^{a}		
Manure		10^{f}	14^{f}	17^{f}	15^{f}	14^{a}		
Means		7^{c}	12^{c}	14^{c}	14^{c}	12		

Treatments exerting a significant effect :

Manure	(a) $P = <0{\cdot}05$
Row distance	(b) $P = <0{\cdot}05$
Plant distance	(c) $P = <0{\cdot}05$
Size	(d) $P = <0{\cdot}01$
Interaction of :	
Manure and row distance	(e) $P = <0{\cdot}05$
Manure and plant distance	(f) $P = <0{\cdot}05$
Row distance and plant distance	(g) $P = <0{\cdot}05$
Plant distance and size	(h) $P = <0{\cdot}05$
Row distance and plant distance and size	(k) $P = <0{\cdot}05$

for the large plants, at a 6 in. plant spacing, thereafter decreasing as the plant spacing increases. With 8 in. and 12 in. row spacings, increasing the plant distances up to 9 in. (6 in. for 16 in. rows) with large plants increases the yield of large bulbs, but for the small plants this is so only for plant distances up to 6 in., in the 8 in. and 12 in. rows and there is no effect with the 16 in. rows. Only at one spacing (8 in. × 3 in.) does the yield of large bulbs, on the " small bulb " plots, fail to equal the maximum values obtained on the " large bulb " plots. These data, therefore, confirm the statements of Hoare (1945) and Stuart (1947) that large daughter bulbs are produced when small bulbs are planted.

The yields of small bulbs are affected by the proportion of the crop riddled out as large bulbs, but reference to Table VI shows no effect of manuring on the yield

TABLE VI.

Yield of small (¾ in.—1¼ in. diameter) shallots (pounds per plot) grown at several spacings and two manurial levels.

Row distance.	Planting material.	Planting distance. 3″	6″	9″	12″	Means.
8 in.	Large	16^{h}	19^{h}	17^{h}	16^{h}	17^{f}
		20^{d}	**17^{d}**	**14^{d}**	**12^{d}**	**16^{a}**
	Small	24^{h}	16^{h}	11^{h}	8^{h}	15^{f}
12 in.	Large	19^{h}	18^{h}	12^{h}	9^{h}	14^{f}
		19^{d}	**14^{d}**	**9^{d}**	**7^{d}**	**12^{a}**
	Small	19^{h}	10^{h}	7^{h}	5^{h}	10^{f}
16 in.	Large	20^{h}	13^{h}	10^{h}	7^{h}	13^{f}
		18^{d}	**10^{d}**	**8^{d}**	**5^{d}**	**10^{a}**
	Small	15^{h}	7^{h}	5^{h}	3^{h}	8^{f}
Manurial treatment						
No manure	Large	16	16	13	11	14^{e}
		19^{g}	**17^{g}**	**13^{g}**	**11^{g}**	**15^{c}**
Manure		21	17	13	11	15^{e}
No manure	Small	20	12	8	6	11^{e}
		19^{g}	**11^{g}**	**8^{g}**	**5^{g}**	**11^{c}**
Manure		19	10	7	5	10^{e}
Means		**19^{b}**	**14^{b}**	**10^{b}**	**8^{b}**	**13**

Treatments exerting a significant effect :

Row distance	(a) $P = <0{\cdot}01$
Plant distance	(b) $P = <0{\cdot}01$
Size	(c) $P = <0{\cdot}01$
Interaction of :	
Row distance and plant distance	(d) $P = <0{\cdot}05$
Manure and size	(e) $P = <0{\cdot}05$
Row distance and size	(f) $P = <0{\cdot}05$
Plant distance and size	(g) $P = <0{\cdot}05$
Row distance and plant distance and size	(h) $P = <0{\cdot}05$

of small bulbs, and no significant interaction of manure × row distance or of manure × plant distance. The yield of small bulbs is greater when large rather than small bulbs are planted, except at the closest spacings, but the results are influenced by the weights below ¾ in. diameter, which were discarded. These weights are appreciable only at the closest spacings on the "small bulb" plots, e.g. 8 in. × 3 in., 4·2 lb.; 8 in. × 6 in., 1·5 lb.; 12 in. × 3 in., 2·3 lb.; and 16 in. × 3 in., 1·0 lb. per plot. At all other spacings they are less than 1·0 lb. per plot. These data serve to indicate the extent to which a lowering of the grading limit might increase the yield of "small" bulbs. Any possible increase from this source is appreciable only at closest spacings, where already the highest yields are indicated. For the large bulb plants, the weights rejected were much greater (e.g. 10·3 lb. on the 8 in. × 3 in. plot) and here a lowering of the grading limit, or an increase in mean bulb weight, might appreciably alter the yield of "small" bulbs.

It was pointed out earlier that an assessment of yield should involve a subtraction from the gross yield of the weight of bulbs planted. More properly, when large bulbs are planted, their weight should be subtracted from the yield of large bulbs, and similarly when small bulbs are planted, their weight must be subtracted from the yield of small bulbs. The results of making these allowances are seen in Table VII. It is clear from this Table that when large bulbs are planted sufficiently closely to give maximum gross yields, they fail to reproduce their own weight of large bulbs. Hence a permanent policy of planting large bulbs closely would be impracticable. Small bulbs at all the spacings tested produce an excess of small

Table VII.

Net yields for large bulb plants. Weight planted is subtracted from weight of large bulbs at harvest.

(In lbs. per plot.)

	Weight of bulbs over 1¼″.					Weight of bulbs between ¾ and 1¼″.			
	Plant distance.					Plant distance.			
Rows.	3″	6″	9″	12″	Rows.	3″	6″	9″	12″
8 in.	−13·1	−3·9	+4·6	+6·4	8 in.	+16·2	+19·2	+16·7	+15·5
12 in.	− 6·9	+3·1	+9·4	+9·4	12 in.	+19·0	+17·5	+11·5	+ 9·2
16 in.	− 3·8	+5·4	+8·7	+10·5	16 in.	+20·2	+13·2	+10·0	+ 7·2

Net yields for small bulb plants. Weight planted is subtracted from weight of small bulbs at harvest.

	Weight of bulbs over 1¼″.					Weight of bulbs between ¾ and 1¼″.			
	Plant distance.					Plant distance.			
Rows.	3″	6″	9″	12″	Rows.	3″	6″	9″	12″
8 in.	+ 7·3	+16·2	+18·3	+18·3	8 in.	+18·0	+12·9	+9·3	+6·7
12 in.	+12·5	+17·7	+18·5	+16·7	12 in.	+15·9	+ 8·3	+5·7	+4·1
16 in.	+16·7	+17·2	+15·7	+16·3	16 in.	+12·4	+ 5·9	+4·3	+2·3

bulbs over the weight planted, and this excess is with the closest spacings almost as great (if not greater) than the total yield of small bulbs from the large bulb plants.

(7) *Plant competition.*

In this experiment, although yield is related to the plant population, the plants which are closely spaced have their growth restricted. It is not possible to state precisely the nature of all the factors which contributed to this competition, but evidence is available that competition for water and for nutrients was important. During dry weather in May, certain plots showed a marked yellowing of the foliage, and on 19th May, a record of foliage colour was made on all plots; the colour categories varied from very yellow (1 mark) to dark green (7 marks), and the results obtained are summarized in Table VIII. The yellowing is seen to be most severe on large bulb plants, and at the close row and/or plant spacings, and was unaffected by manuring. The presumption is that the yellowing is the result of a competition for soil water which is most severe when the plants are closely spaced. Observations made on 15th June, 1948, after a few days of heavy rain, showed that the spacing effects persisted, but a response to manuring became evident, the mean rating for the manured plots being 6·1 and for the unmanured 4·9.

In the absence of any visible response to manuring by 22nd May, it would appear probable that early in the season interplant competition was mainly for soil water, and after the onset of wet weather in early June, for nutrients. That the close spacing resulted in competition for some constituent or constituents of the fertilizer mixture applied to the manured plots is further suggested by the fact that the response to manuring was greatest on the closely spaced plots.

TABLE VIII.

Average colour, in categories, of foliage of shallots grown at several spacings. Recorded 19th May, 1948.

	Planting distance.				
Row distance.	3″	6″	9″	12″	Means.
8 in.	2·1	3·6	5·1	5·3	4·0
12 in.	2·8	4·5	5·7	5·9	4·7
16 in.	4·1	5·4	5·9	6·6	5·4
Planting material.					
Large	1·9	3·2	4·7	5·3	3·8
Small	3·7	5·9	6·4	6·6	5·7
Means	2·9	4·6	5·6	6·0	

1 = Very yellow. 7 = Dark green.

SUMMARY.

(1) Dutch yellow shallots of two sizes (over 1¼ in. diameter, 20 per lb., and less than 1¼ in. diameter, 53 per lb.) were planted at three row distances and four plant distances, at two manurial levels, on a fertile loam. The mean yield obtained was at the rate of 11 tons 10¾ cwt. per acre.

(2) Close spacing and the planting of large bulbs increased susceptibility to drought, as indicated by foliage yellowing.

(3) Close spacing accelerated ripening.

(4) The number of daughter bulbs per plant was greatest when large bulbs were planted but the number was reduced slightly by close spacing.

(5) Bolting was appreciable only in the plants from large bulbs and its incidence was decreased by close spacing.

(6) The total yield was increased (but barely significantly) by manuring, the manurial response being greatest at the close row and plant spacings.

(7) Large bulb plants tended to out-yield small bulb ones at wide but not at close spacings.

(8) When allowance is made for the weights of bulbs planted, at the spacings giving the highest net yields (8 in. rows or 3 in. plant spacings), small bulb plants out-yield large ones, and the response of the small bulb plants to manuring is greatest at the close spacings. The differential response to manuring accounts for much of the increased yield of the close row and close plant plots over the wide row and wide plant ones (with small bulb plants).

(9) The mean bulb weight of daughter bulbs is greatest in the plants obtained by planting small bulbs and increases for every increase in the row or plant distance.

(10) Separation of the crop into three size grades suggests that when the necessity of replacing planting material is taken into account, maximum yields of large bulbs are obtained by planting small bulbs so as to allow not less than 48 sq. in. per plant. For maximum yields of small bulbs close planting of small bulbs in close rows is necessary.

ACKNOWLEDGMENTS.

My best thanks are due to Professor Ashby, who placed facilities for this work at my disposal, and to Professor Bartlett for advice on the proper statistical treatment of the results. Assistance was given by Miss Dickenson and Mr. P. Chambers in the planting, and by Messrs. Petford, Hough and Laycock in the harvesting of the experimental material. Mr. F. Wallen efficiently supervised all the necessary cultivations.

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(*Received* 23/9/50.)

SPACING EXPERIMENTS ON VEGETABLES

IV. THE YIELD OF GLOBE BEET GROWN AT TWELVE SPACINGS AND TWO MANURIAL LEVELS, IN CHESHIRE, 1948

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INTRODUCTION.

SPACINGS for Globe Beet suggested in recent writings include variations in the row distance from 12 in. (Ministry of Agriculture) to 24 in. (Hoare, 1945), and in the thinning distance from 2 in. (for 15 in. rows) to 6 in. (Hoare, 1945) or 8 in. (Ministry of Agriculture, 1942), giving a range of population densities of from 9 to 43 per sq. yard assuming that a full stand of plants is obtained. Although the row spacing employed may, in fact, be decided mainly on the score of ease of cultivation and the stand of plants in the row affected by the germination, it is noticeable that recommendations as to spacing are not, as a rule, accompanied by any suggestions as to the extent to which yield may depend on the plant population. It is recognized, however, that wide spacings of rows or plants may lead to the production of over-sized roots (Johnstone, 1948). Further, we have no information as to how nearly we may expect the actual plant population to approach the " theoretical " number based on the nominal thinning distances employed.

In the experiment which is to be described, Globe Beet were grown at four row spacings, at three thinning distances, and at two manurial levels. The effects of these factors on yield were investigated.

GENERAL EXPERIMENTAL PROCEDURE.

THE experiment was carried out at the University of Manchester Jodrell Bank Experimental Station, Lower Withington, Cheshire, in 1948 on a heavy loam of high fertility. The site was winter ploughed to a depth of 8 in., limed in February at the rate of $1\frac{1}{4}$ tons per acre, and fertilizers applied (where necessary) in early April and harrowed in.

The effects of the following treatments were investigated :

(1) Manures. (*a*) No added fertilizer.
(*b*) 10 cwt. per acre of a mixture of superphosphate of lime, 2 parts, sulphate of ammonia, 2 parts, and sulphate of potash, 1 part.
(2) Row spacings : 9 in., 12 in., 18 in. and 24 in.
(3) Three nominal thinning distances : 2 in., 4 in. and 6 in.

These spacings gave a range of populations from approximately 51,000 to 240,000 plants per acre compared with the " theoretical " values for the spacings employed of 43,560 to 348,480.

The experiment was arranged in three randomized blocks. The main plots, consisting of the combinations of manure and row spacings within each block were split for the three thinning distances (Snedecor, 1946), to give a total of 72 plots, each of 72 sq. ft.

Seed of the variety " Crimson Globe " (Caldwell) was sown by hand on 26th April, 1948. Germination was good, and the plants grew satisfactorily, although slowly, during dry weather in May and early June. The plots were thinned on 31st May and 5th June. Good quality labour was available for the thinning, and for this operation the men were provided with canes marked at appropriate distances. After rain in early June, all the plants grew away rapidly. Throughout the remainder of the season, rainfall was abundant and the plants probably never suffered from a water shortage. Counts (not given) of the plants present in mid-June indicated a considerable deficiency of plants on all plots thinned to a nominal distance of 2 in., but a close approximation to the expected number of plants on all the other plots, and an increase in the plant number due to manuring.

Harvesting was commenced on 3rd September and was concluded on the 10th September, 1948. At harvest, data were collected for (1) number of plants per plot, (2) number of bolters per plot, (3) total weight of roots (cleaned but not washed) per plot, (4) number and weight of roots of $1\frac{7}{8}$ in. diameter or over (" large " roots), excluding bolters, per plot, and (5) weight of tops per plot.

At harvest the plots on which the nominal thinning distance was 2 in. were noticeably less weedy than the 4 in. and 6 in. plots. The weeds present on all the plots at harvest were mainly *Poa* spp. and appeared to originate either in or very close to the row, cultivation having prevented weed establishment in the inter-row space. Presumably the close stand of plants on the 2 in. plots effectively prevented much weed growth in the rows, whereas weed growth was not prevented when the stand of plants was less full (as on the 4 in. and 6 in. plots).

RESULTS.

(1) *Number of plants per plot.*

Table I shows the total number of plants present per plot at harvest. The numbers showed a mean increase of 19 plants per plot over the June counts. This is probably accounted for by some delayed germination, and by counting in June, as a single plant, two plants left at the same station. At the 2 in. and 4 in. thinning distances the number of plants present falls short of the expected number. This deficit is greatest in the 2 in. plots, where only 74 per cent. of the expected number of plants was obtained. At the 6 in. distance there were rather more plants than the expected number. Manuring increased the stand of plants.

(2) *Bolting.*

Three per cent. of the plants bolted. This proportion was not affected by any of the treatments investigated. Bolters are included in the figures for total yields, but the roots of bolters are not included in the weights of " large " roots.

(3) *Total yield of roots.*

The total yields of roots (cleaned but not washed) harvested per plot are shown in Table I. This yield is significantly increased by manuring, and decreases for every increase in the row spacing and thinning distance. The interactions, manure × thinning distance and row distance × thinning distance are both significant, the increase due to manuring and the decrease due to increasing the row distance both

TABLE I

Showing, for Globe Beet, the number of plants per plot at harvest and, in black type, the total yield of roots in pounds per plot.

Row distance.	Thinning distance.							
	2"		4"		6"		Means.	
9 in. ..	396	**76**[e]	264	**75**[e]	185	**65**[e]	282	**72**[b]
12 in. ..	330	**69**[e]	210	**72**[e]	159	**63**[e]	233	**68**[b]
18 in. ..	214	**70**[e]	128	**62**[e]	108	**58**[e]	150	**62**[b]
24 in. ..	166	**56**[e]	109	**56**[e]	85	**50**[e]	120	**54**[b]
Manurial treatment								
No manure ..	263	**61**[d]	162	**57**[d]	125	**56**[d]	183	**58**[a]
Manure ..	291	**75**[d]	193	**73**[d]	143	**62**[d]	209	**70**[a]
Means ..	277	**67**[c]	178	**65**[c]	134	**59**[c]	196	**64**

Treatments which significantly affect the yield of roots:

Manure	(a)	$P = <0{\cdot}05$
Row distance	(b)	$P = <0{\cdot}01$
Thinning distance	(c)	$P = <0{\cdot}05$
Interaction of:		
Manure and thinning distance	(d)	$P = <0{\cdot}05$
Row distance and thinning distance	(e)	$P = <0{\cdot}05$

being least at the greatest thinning distance employed. It is clear from this Table that maximum yields of roots are not obtained if the thinning distance exceeds 4 in. for 9 in. and 12 in. rows, or if the row spacing exceeds 18 in. For maximum yields of root, therefore, it is necessary either for the rows to be closely spaced, or for the plants to be closely spaced within the rows.

The effects of manuring are complex, and will be discussed later.

(4) *Yield of "large" roots.*

Interest centres mainly, not on the total number or weight of roots, but on the number and weight that attain a usable size. In this work, roots of a diameter of less than $1\frac{7}{8}$ in. were discarded as being too small to be of value, and Table II shows the number and weight of roots of a diameter of $1\frac{7}{8}$ in. and over.*

From this Table it is seen that for every increase in the spacing over 9 in. × 2 in. the number of saleable roots decreases, but the weight of "large" roots obtained at 9 in. × 2 in. does not differ significantly from the yields for the 9 in. × 4 in., 9 in. × 6 in., 12 in. × 2 in., 12 in. × 4 in. and 18 in. × 2 in. plots.

Beyond these limits the yield falls, suggesting that for maximum yields (weights) of large roots not less than 112,000 plants (arranged in 9 in., 12 in. or 18 in. rows) per acre is desirable. Maximum yields, if obtained with fewer plants than this, will result in the production of undesirably large roots.

Dependence of the yield of large roots on the population density within the row increases as the row spacing increases up to an 18 in. row distance. With closely spaced rows a relatively poor stand of plants affects the yield but little.

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* Compared with a minimum diameter of 2 in. subsequently recommended by Ministry of Agriculture (Marketing Leaflet, No. 102, 1948).

TABLE II.

Showing number and, in black type, weight (pounds) per plot of " large " Globe Beet for several spacings and two manurial levels.

Row distance.	Thinning distance.						Means.	
	2"		4"		6"			
9 in.	268	**66**^{d}	205	**69**^{d}	153	**61**^{d}	209	**66**^{b}
12 in.	224	**61**^{d}	175	**70**^{d}	127	**59**^{d}	175	**63**^{b}
18 in.	169	**65**^{d}	108	**53**^{d}	86	**53**^{d}	121	**57**^{b}
24 in.	132	**52**^{d}	89	**52**^{d}	71	**48**^{d}	97	**50**^{b}
Manurial treatment								
No manure	191	**56**^{e}	136	**54**^{e}	105	**52**^{e}	144	**54**^{a}
Manure	205	**66**^{e}	153	**68**^{e}	113	**58**^{e}	157	**64**^{a}
Means	198	**61**^{c}	144	**61**^{e}	109	**55**^{e}	150	**59**

Treatments significantly affecting yield of " Large " roots :
Manure (a) P = <0·05
Row distance (b) P = <0·05
Thinning distance (c) P = <0·05
Interaction of :
Row distance and thinning distance (d) P = <0·05
Manure and thinning distance (e) P = <0·05

With rows spaced at 18 in., a good stand of plants is essential if a high yield is to be obtained. Close spacing of the rows is inconvenient, because of the difficulties which arise in carrying out the inter-row cultivation necessary for weed control. The experiment, therefore, emphasizes the desirability of evolving methods of weed control readily applicable to closely spaced rows of beet.

Adoption of the size limits (2 in. to 3½ in. diameter) subsequently recommended by Ministry of Agriculture (Marketing Leaflet No. 102, 1948), i.e. raising the minimum diameter of " large " roots from $1\frac{7}{8}$ in. to 2 in., would, it is expected, have affected most adversely the yields of large roots at the closest spacings, but it is not possible to estimate the magnitude of this effect. None of the roots of $1\frac{7}{8}$ in. diameter or over, however, proved unacceptable to the market because of their small size, but roots of a diameter of 3·3 in.* (11·3 oz.) or over were unsaleable. Losses from this cause may be appreciable on the widely spaced plots as the mean weight of " large " roots from the 24 in. × 6 in. plot (where the yield was low) was 10·8 oz. Hence close row or plant spacing is desirable not only as a means of increasing yield, but as a method of restricting root size.

(5) *Effects of manure on yield and growth of roots.*

It is now possible to assess the effects of the manurial treatment on root growth and yield. Manuring increases the mean number of plants per plot by 14 per cent. and increases the mean yield of roots per plot by 20·7 per cent. (12 lb.), from which it would appear that the main effect of manure on yields is through its effect on the plant population. For the unmanured plots the regression of plant population on yield of roots can be calculated and total yield of roots per plot (in lbs.) is given by $16\cdot5\ (N)^{0\cdot244}$ where N is the number of plants per plot. Using this formula we can then calculate the yield expected (in the absence of manuring) for the plant populations present on the manured plots. When this is done, we find that of the mean gain of 12 lb. per plot caused by manuring, 2 lb. only is due to the increase in plant population, and 10 lb. is due to the increased growth of the plants, caused by manuring. This effect of the manure in increasing yield by increasing the plant growth is least at the wide plant and row spacings, being 12 lb., 12 lb., 12 lb. and 3 lb. for the 9 in., 12 in., 18 in. and 24 in. rows respectively, and 9·5, 13·5 and 4·5 lb. for the 2 in., 4 in. and 6 in. thinning distances. It may well be, of course, that as at the wide row and plant spacings the number of plants present is small, the capacity for an increased yield brought about by increased root growth is limited.

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* Compared with a maximum diameter of 3½ in. subsequently recommended by the Ministry of Agriculture (Marketing Leaflet No. 102, 1948).

(6) *Yield of tops.*

The yield of tops in pounds per plot is shown in Table III. Manuring causes an increase in top growth, and this increase is much greater relatively than the increase in the yield of roots caused by manuring. The effects of altering the thinning distance are greater on top growth than on root growth, but the effects of altering row distance are similar relatively to the effects on root yield. Clearly, the effects of manure on top growth are almost entirely direct and only a very small proportion of the increase can be due to the effects of manuring on plant number. This manurial effect is least at the wide row and plant spacings. Similarly, the effects of altering the row distance decrease as the thinning distance increases.

TABLE III.

Showing yield of tops (pounds per plot) and, in italics, the root top ratio for Globe Beet at several spacings and two manurial levels.

	Thinning distance.							
Row distance.	2″		4″		6″		Means.	
9 in. ..	35	*2·19*	34	*2·29*	24	*2·84*	31^{b}	*2·45*
12 in. ..	38	*1·96*	31	*2·51*	27	*2·50*	32^{b}	*2·32*
18 in. ..	34	*2·19*	24	*2·47*	24	*2·61*	27^{b}	*2·43*
24 in. ..	28	*2·02*	24	*2·39*	20	*2·58*	24^{b}	*2·33*
Manurial treatment								
No manure ..	27^{d}	*2·30*	22^{d}	*2·68*	19^{d}	*2·97*	23^{a}	*2·65*
Manure	40^{d}	*1·87*	35^{d}	*2·16*	28^{d}	*2·29*	34^{a}	*2·11*
Means	34^{c}	*2·01*	28^{c}	*2·36*	23^{c}	*2·49*	28	*2·39*

Treatments significantly affecting the yield of "tops":

Manure	(a) $P = <0\cdot01$
Row distance	(b) $P = <0\cdot05$
Thinning distance	(c) $P = <0\cdot01$
Interaction of:	
Manure and thinning distance	(d) $P = <0\cdot05$

DISCUSSION.

In so far as the data which have been presented have a practical value, the conclusions are clear: to obtain the maximum number of beet of a usable size, close spacing of the plants in close rows is essential. The highest number of saleable beet per unit area was, in these experiments, obtained with the closest row and plant spacings, i.e. 9 in. rows and a 2 in. nominal thinning distance, giving 4·13 plants per foot of row (approximately 240,000 plants per acre). To obtain the maximum weight of large roots, either close spacing of the rows, or close spacing of plants in rows not more than 18 in. apart to give not less than 112,000 plants per acre, appears desirable. Row spacings are more easily controlled than plant spacings, but close rows present special problems in inter-row cultivation. It is doubtful if any real advantage accrues from a row spacing of less than 12 in., although as the mean root weight is considerably increased by an increase in the row spacing from 9 in. to 12 in., the risk of producing roots which are undesirably large is increased, especially if for any reason (e.g. irregular germination), many of the plants in the row are widely spaced. The results of this experiment suggest that minor irregularities in the row are of little importance, when the rows are closely spaced. If, however, the row spacing is increased to 18 in., as it may be to facilitate mechanical inter-row cultivation, a close stand of plants is essential, both in order to maintain the yield and to restrict root size. There is no evidence that any particular arrangement of the plants is especially advantageous, provided a sufficient number of plants are present to give a maximum yield.

These results were obtained in an experiment where the mean yield of large roots was at the rate of 14 tons 2 cwt. per acre, but for the five most desirable spacings was at the rate of 15 tons 16 cwt. per acre. These higher yields were clearly not susceptible to further increase by increasing the plant population. Hence it is presumed that yields higher than those obtained here can be obtained only by increased growth of the plants, and this, unless either rows or plants are closely spaced, is likely to result in many of the roots becoming undesirably large. It is clear, therefore, that when experience has shown that yields of this magnitude are possible, close row or close plant spacing is essential if these high yields are to be obtained without the production of unduly large roots.

The experiment, on the whole, throws little light on the nature of inter-plant competition, with closely spaced plants. When allowance is made for the effect of manuring on the number of plants present, it is found that the increase in mean root weight due to manuring is greatest absolutely, but least relatively, when the area available per plant is greatest, i.e. when the competition is least severe, but the increase in the total yield of roots due to manuring tends to be highest at the closer spacings. This does suggest that the restriction in size which results from close spacing may, in part, be due to a competition for nutrients. Competition for water is unlikely to have been important. From mid-June onwards rainfall was abundant, and in another experiment variations in the plant population from 1·5 to 2·5 per foot of row (with 18 in. rows) were without effect on root size by the end of June (Warne, 1951a). Hence it is probable that the competition effects leading to a restriction in root size at the close spacings, operated subsequently to June, i.e. during a period of abundant rain.

In another experiment, too, it was found for globe beet that reducing the thinning distance reduced the value of the root/top ratio (Warne, 1951b). Moreover, this ratio increases as the season progresses (Warne, 1951a), so that these results were not incompatible with the view that close spacing retards growth and also delays the development of physiological maturity. The root/top ratios have been calculated for this experiment and the values obtained are incorporated in Table III. It is seen that manuring and decreasing the thinning distance both decrease the value for this ratio, but that altering the row distance is without effect.

Manuring, therefore, has an effect similar to that of reducing the thinning distance. This might be explained if one of the effects of one or more of the manurial constituents was to delay the seasonal change in the root/top ratio. It may be, however, that the production of an increased amount of foliage per unit length of row, whether due to manuring or to close spacing of the plants, leads to a decrease in leaf efficiency, so that deposition of carbohydrate in the roots is reduced, and root growth retarded. Close spacing of the plants and manuring do both cause an increased production of leaf per unit length of row, and so long as we consider only the effects of manure and of altering the thinning distance, there is a general negative correlation between weight of foliage per plot and the root/top ratio, suggesting that leaf efficiency may be negatively correlated with the amount of foliage present.

These effects occurred during a period of abundant rainfall, and it is unlikely that they were caused by a water shortage, but they might result from mutual shading of the foliage, reducing the amount of photosynthesis. Manuring decreases the value of the root/top ratio at every row distance; but increases in the row distance, whilst they result in a decrease in the total weight of tops per plot, cause an increase in the weight of tops per unit length of row, but do not affect the root/top ratio. This would be so if a decrease in leaf efficiency due to crowding of leaves in the row was counterbalanced by an increased efficiency due to better illumination of the leaves of the plants in the widely spaced rows. It is not, however, possible as a result of this experiment, to state precisely the nature of the inter-plant competition, but the spacing effects observed are most readily explained as being the consequence of competition for nutrients, and of mutual shading at the closest spacings.

SUMMARY.

(1) Globe Beet were grown at four row distances (9 in., 12 in., 18 in. and 24 in.), and three thinning distances (2 in., 4 in. and 6 in.) and two manurial levels (no manure, and 10 cwt. of "complete" fertilizer per acre) on a fertile loam, and gave a mean yield of 14 tons 2 cwt. per acre.

(2) Manuring increased the stand of plants, the total yield of roots, and the yield (weight) of "large" roots. The response to manuring was greatest at the closest spacings.

(3) The maximum number of "large" roots was obtained with the closest spacing employed (approximately 240,000 plants per acre), and the maximum weight of large roots with 112,000 or more plants per acre.

(4) Within the spacing limits employed no particular arrangement of the plants was especially advantageous.

(5) The effects of close spacing in restricting plant growth in this experiment were most likely due in part to competition for nutrients, accompanied by a mutual shading of the foliage.

ACKNOWLEDGMENTS.

I wish to thank Professor Ashby for placing at my disposal the facilities for this work. Acknowledgments are due also to Mr. F. Wallen, who supervised the cultivation of the experimental crop, and to Messrs. Petford and Hough, who were responsible for the harvesting.

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(*Received* 23/9/50.)

VERTICILLIUM WILT OF THE STRAWBERRY

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Verticillium wilt of the Strawberry was first studied by Thomas (1932) in California. He isolated the pathogen, which he ascribed to the species *Verticillium albo-atrum*,* and demonstrated its pathogenicity by soil inoculation experiments. He observed a striking correlation between the occurrence of wilt in strawberries in commercial plantations and the planting of tomatoes, a crop which is also susceptible to *Verticillium* wilt. Thomas reported that some varieties of strawberry (e.g. Marshall) appeared more resistant under field conditions than others (e.g. Nich Ohmer). The main control measure recommended was the avoidance of land on which other susceptible crops had been grown, and it was also suggested that the disease might be spread by planting affected runners. Thomas noted a previous record of the isolation of *Verticillium* from strawberry by Van Poeteren (1929) in Holland. Van Poeteren believed the fungus (possibly *V. albo-atrum*) to be the cause of a disease but performed no inoculation experiments.

Darrow and Waldo (1941) reported the disease to be still prevalent in central California, sometimes causing losses of up to 75 per cent., especially after previous cropping with tomato, potato and raspberry.

The first record of the disease in England is given in a report from the Cambridge Horticultural Station (1932) which states that severe losses were caused in the King's Lynn district and at Cambridge. A species of *Verticillium* was constantly isolated from affected plants but no further work was described.

Wormald and Harris (1938) reported isolations of *V. dahliae* from wilted Huxley and Royal Sovereign strawberries in Kent, and Isaac (1949) obtained infection of plants of Royal Sovereign variety by inoculation with *V. albo-atrum* and with *V. dahliae* from other hosts, and noted that the former was the more pathogenic.

The present writers started their study of this disease in 1944 in continuation of the preliminary studies by Harris and Isaac.

FIELD SURVEYS.

Field observations were made in 1946 and 1947 in several strawberry areas, especially the Wisbech and King's Lynn district, where many plantations were affected in varying degrees. The disease had its greatest effect in the first year, causing up to 50 per cent. loss of plants in some fields. As runners are usually grown from one-year-old plants, the attack of wilt disease often causes considerable loss of new planting stock. In this area, however, runners are usually allowed to fill the spaces in the row between the parent plants, thus producing a "matted row" for cropping in the two or three subsequent seasons. Where this had been done a fairly good stand of plants was obtained in the second season even from badly affected fields, as plenty of runners were usually available from nearby plants to fill the gaps caused by the disease. In consequence, where the disease was not too severe in the first season, its effect on the subsequent crop of fruit was apparently very small.

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* *Note.* The species isolated by Thomas was probably that known to the present writers and others as *V. dahliae*. The distinctions between *V. dahliae* and *V. albo-atrum* are discussed later.

The disease was of greatest economic importance in Special Stock runner beds of the Huxley and Oberschlesien varieties grown for the production of selected stock with minimal virus infection. In such nurseries the parent plants are widely spaced and, to facilitate the rogueing of virus-infected plants, the runners are grown in isolated blocks with alleys between them (Rogers and Buttfield, 1943). Thus, as runners from plants in one block cannot grow into the spaces created by the death of a plant in another block, the losses from wilt are liable to be much greater than in an ordinary runner bed. In several nurseries seen from 1945 to 1947 the disease caused a reduction of up to 75 per cent. in the crop of runners and some were abandoned for this reason (see Fig. 1).

The infected plants in most outbreaks were fairly evenly distributed over large areas of the field and no clear evidence was obtained of any spread from infection foci. The disease was reported by growers and others to be more severe in some seasons than in others, but the causes of this phenomenon are obscure.

SYMPTOMS.

The symptoms of strawberry wilt are rather variable. Observations on the variety Huxley in commercial plantations and on plants infected by soil inoculation gave the following information.

The first signs of wilt in maiden plants may be seen from late June or early July onwards until September or October. One or more of the outer leaves assumes a grey-green slightly silvery appearance, or sometimes has a reddish colour. Bluish black lesions appear on the petioles of such leaves, the laminae become gradually dry and brown and the leaves eventually lie flat on the ground. At this stage the base of the petiole is noticeably rotted. As the disease progresses, more leaves wither in this way until, in a few weeks' time, all the older leaves have died. The symptoms on the older leaves are often accompanied by some yellowing of the young leaves, and as the disease progresses any new leaves produced are usually small, yellowish in colour and distorted. Symptoms may remain at this stage throughout the season, producing a stunted plant, or progressively more leaves may die, so that eventually the whole plant dies. In one experiment in which the plants were grown on soil infected with *V. albo-atrum*, the first symptom, noted in June, was a pronounced yellowing of the central leaves. This preceded the more usual death of the outer leaves by about three weeks and was not seen on the control plants.

The stolons on infected plants develop lesions similar to those on the petioles and often several inches long. These eventually become necrotic and completely girdle the stolon, causing any unrooted runners on the stolon to die from lack of moisture, often after showing incipient wilt symptoms. Symptoms may also appear on those runners which are already rooted in the ground but a varying proportion of these may survive.

If the crown of an infected plant is cut transversely a dark brown discoloration of some of the vascular bundles may be noted. This symptom is not invariably seen, however, and as other organisms may cause a similar discoloration it is not a good diagnostic feature. The roots of infected plants are often somewhat decayed, but present no feature which serves to distinguish the disease from others which cause root lesions. There is no obvious discoloration of the central stele, however, as is present with Red Core Disease caused by *Phytophthora fragariae*.

In the writers' experience, the most characteristic symptom is the death and the prostrate appearance of the outer leaves which, on one-year-old plants, generally serves to distinguish the disease from all others. Figs. 2 and 3 illustrate typical mildly and severely affected plants of the variety Huxley.

Symptoms are much less obvious on two- and three-year-old plants. If the plant survives an attack of wilt in its first year, it may appear fairly normal in the second year with only slight desiccation of the outer leaves, which is difficult to distinguish from the appearance of normally senescent leaves.

STUDY OF THE PATHOGENS.

The nomenclature of the genus *Verticillium* is somewhat confused, due mainly to differing interpretations by various workers of the description of *V. albo-atrum* by Reinke and Berthold (1879). In this description *V. albo-atrum* was stated to form resting mycelium in the vascular tissue of the potato. Dark-walled torulose cells were produced and when neighbouring hyphae were contiguous the groups of cells formed sclerotia. In the absence of cultural studies, information on the structure of these sclerotia depends entirely on the description and drawings made by Reinke and Berthold of sections of diseased potato tissue.

Klebahn (1913) described a form of *Verticillium* which in culture produced microsclerotia by the irregular septation and budding of the cells of single hyphae. He considered this form to be distinct from *V. albo-atrum* as described by Reinke and Berthold and he named it *V. dahliae*. The separation of this species received support from Van der Meer (1925) and other workers but others, notably Rudolph (1931), used the name *V. albo-atrum* for the microsclerotial form on the grounds that Reinke and Berthold worked with this form. Rudolph regarded the dark mycelium form as a variety of *V. albo-atrum* and called it *V. albo-atrum* var. *tuberosum*. A few workers, notably Presley (1941, 1950) do not regard these two forms as distinct.

The literature on this subject has been recently summarized by Isaac (1949), who also showed that the two forms could be distinguished by their physiological and pathological characters as well as by their morphology. Isaac supported the view that the microsclerotial form should be named *V. dahliae* and the dark mycelium form *V. albo-atrum*. This view is also held by the present writers.

Thomas (1932), in referring to the pathogen he isolated from wilted strawberries as *V. albo-atrum*, stated that it was indistinguishable from the fungus described under that name by Rudolph, and causing " blue-stem " of raspberry. It is thus probable that it was the fungus known to the present writers as *V. dahliae*.

In the present study all the isolates of *Verticillium* obtained from wilt-infected strawberries could readily be assigned to one or other of the species *V. albo-atrum* or *V. dahliae*. No intermediate forms were found.

Specimens of wilted strawberries were obtained during 1945-47 from 27 localities, mainly in Norfolk, Suffolk and Kent, either in the course of the field surveys or through advisory officers and others. Of these outbreaks, five were apparently caused by *V. albo-atrum*, and twenty by *V. dahliae*. Two outbreaks yielded isolates of both fungi. Although no accurate assessment could be made of the relative prevalence of the two species, a definite impression was gained that *V. dahliae* was more prevalent than *V. albo-atrum*. No differences were noted in the severity of the outbreaks caused by each species, but it was impossible to assess this satisfactorily owing to the lack of uniformity of soil conditions, etc., among outbreaks.

The pathogen was usually isolated by cutting out a portion of the crown about ½ to ¾ in. long, stripping off the leaves and then surface-sterilizing it by dipping in alcohol and flaming. It was then cut longitudinally and portions of vascular tissue transferred to prune agar or potato dextrose agar (including 1 per cent. potassium thiocyanate to reduce bacterial growth) in petri dishes. At the same time, transverse sections of the crown about ¼ in. thick were incubated, without prior surface sterilization, in moist petri dishes. The isolates on agar had usually grown to a sufficient size for identification (about 2 cm. diameter) in eight to ten days. Other isolates could be obtained from the spores of *Verticillium* present on the tufts of mycelium which usually grew from the vascular bundles of the sections of the crown incubated in moist dishes. Isolates were also obtained from necrotic portions of stolon, petiole and leaf lamina.

Although isolation of the pathogen directly on to agar provided the quickest means of identification of the species concerned, it was found to be not wholly satisfactory. Many bacterial contaminants grew from the vascular tissue, and occasionally other fungi (notably *Gliocladium roseum*, *Cephalosporium* sp., and *Penicillium* spp.). Somewhat conflicting results were also obtained on occasions, plants which were apparently infected yielding no isolate of any sort. While such results may, of course, have been due to incorrect diagnosis, it seems more likely that the methods of isolation were sometimes at fault. Owing to the localization of the pathogen, uninfected portions of tissue may have been used and the sterilization methods may have killed the fungus when it was present only near the surface.

INOCULATION EXPERIMENTS.

1. *Isolates from strawberry.*

Isolates of *V. albo-atrum* and *V. dahliae* obtained from wilted strawberries were grown for six weeks on sterilized chopped hop stem. Thirty strawberry runners obtained from normal plants of the Huxley variety were planted in a plot of ground in early April. Before planting, 15 gm. of the *V. albo-atrum* inoculum were put into each of seven of the planting holes and 15 gm. of *V. dahliae* inoculum in seven of the other holes. The remaining sixteen holes received no inoculum. The plants were observed throughout the season and finally dug up at the end of November.

Six of the plants inoculated with *V. albo-atrum* showed symptoms of wilt from early July onwards. Five of those inoculated with *V. dahliae* showed symptoms. None of the controls showed any wilt symptoms.

In November isolations were made from the crowns of all the plants. *V. albo-atrum* was isolated from all the six wilted plants inoculated with this fungus, but not from the plant showing no symptoms. Three of the five wilted plants inoculated with *V. dahliae* yielded this fungus. No *Verticillium* was obtained from any of the controls.

2. *Isolates from hops.*

Isolates of *V. albo-atrum* and *V. dahliae* obtained from hop were used in a second inoculation experiment with the Huxley variety of strawberry. The method of inoculation was the same as that already described, but 15 plants were inoculated with a strain of *V. albo-atrum* causing mild (fluctuating) wilt in hops, 15 plants with a strain of *V. albo-atrum* causing severe (progressive) wilt in hops and 15 plants with *V. dahliae*.

Typical wilt symptoms were shown by all the plants inoculated with the "fluctuating" strain of *V. albo-atrum*. Eleven plants inoculated with the "progressive" strain and five plants inoculated with *V. dahliae* showed symptoms. Isolates were subsequently made from four plants showing symptoms in each series and the pathogens were re-obtained. None of the control plants showed any symptoms nor was any *Verticillium* obtained from them.

The results of the inoculation experiments suggest that *V. albo-atrum* is more pathogenic than *V. dahliae* to the Huxley variety of strawberry. This is in agreement with the conclusions of Isaac on his inoculations of the Royal Sovereign strawberry variety. The second experiment described here, however, suggests that strains of *V. albo-atrum* may differ in pathogenicity to strawberry. It is also known from field observations that *V. dahliae* can cause very severe outbreaks of wilt and thus no rigid conclusions can be drawn on the comparative effect of the two fungi in the field.

TESTS OF RUNNERS FROM WILT-INFECTED PLANTS.

As already noted, Thomas (1932) suggested that strawberry wilt might be transmitted to previously clean ground by planting infected runners. Preliminary experiments by Isaac (unpublished) in 1945 suggested, however, that runners from diseased plantations would grow into normal plants, and the writers therefore started a series of experiments in 1946 to test this further.

Runners were collected from three special stock nurseries of the Huxley variety. One was at East Malling and had suffered from a very severe wilt outbreak in 1945, mainly caused by *V. dahliae*. The second was in Suffolk and was similarly diseased, the pathogen in this instance being mainly *V. albo-atrum*, although some *V. dahliae* was also present. Nursery No. 3, situated near Canterbury, was practically free from wilt. In each of the two wilt-affected nurseries 160 runners were collected from badly diseased plants and 160 from slightly diseased plants. Three hundred and twenty runners collected from the healthy nursery were taken from vigorous, apparently healthy plants.

The runners from the wilt-affected nurseries were selected as far as possible at random within each of the two categories, only badly wilted ones being rejected. There were, however, fewer surviving runners on the badly diseased plants than on those not so severely affected. In consequence those in the former category had developed somewhat better than the others. Previous isolation tests had demonstrated that the pathogens were present in many of the runners from each of the two affected nurseries and apparently absent from those collected from the healthy nursery. It was not possible, however, to assess accurately the relative prevalence of disease in the runners in each category and the experiment was thus regarded as a commercial test of runners selected on a visual basis rather than a test of runners known to contain *Verticillium*.

The runners from the healthy nursery were divided into two batches of 160 to provide two sets of control plants, making a total of six categories (see Table I). Forty plants of each category were planted on each of four sites as follows :

(*a*) A plot on a medium loam soil at East Malling, on which red currants had been grown for seven years and which was believed to be free from wilt.

(*b*) A plot of heavy clay loam soil near Paddock Wood, Kent, which had previously been permanent pasture and was believed to be free from wilt.

(*c*) The site of the East Malling nursery which had been severely affected with wilt in 1945. The soil was a medium loam.

(*d*) The site of the severely affected Suffolk nursery—a heavy silt loam.

In each of the four experiments the six groups of forty plants were arranged in four randomized blocks (i.e. each block containing six ten-plant plots). Single guard rows of healthy plants were planted at the sides and ends of each experiment.

The runners were collected in the autumn of 1945. Sites (a) and (c) were planted in November, 1945, and the other two in February and March, 1946.

Wilt incidence was studied on all sites during 1946. In 1947 the plant height and spread measurements and crop weights were recorded on plots (a) and (c).

1. *Wilt incidence* (*1946*).

On site (b) no wilt was seen at any time during the season. On the other sites a final record of wilt was made in late September or early October, the previous records being used for checking this final assessment. Symptoms were classified into three categories—Severe Wilt (W+) when the plants were dead or nearly so; Wilt (W) if distinct symptoms could be seen involving death of the outer leaves and stunting and yellowing of the young leaves; and Doubtful Wilt (W?), if some of the old leaves were dead but the remainder of the plant appeared normal, or if the plant was stunted but not showing clear symptoms. The 1946 results from sites (a) (c) and (d) are given in Table I.

Statistical analysis showed that, whereas the wilt incidence on the sites differed greatly, there was no significant difference within each site between the runners from the six different sources in wilt incidence or possible incipient symptoms. It is thus clear that the performance of the runners was unrelated to the disease status of their parent plants. It was obviously greatly influenced, however, by the disease

TABLE I.

Wilt records on sites (*a*), (*c*) *and* (*d*).

Source of runners.			Site (a) E.Malling "clean" soil.				Site (c) E.Malling infected soil.				Site (d) Suffolk infected soil.			
Site.	No.	Disease status of parents.	No.	W?	W	W+	No.	W?	W	W+	No.	W?	W	W+
East Malling (a)	1	Severely affected	40	0	1	0	38	11	18	1	40	1	0	0
	2	Mildly affected	40	1	1	0	37	8	18	4	40	4	2	0
Suffolk (c)	3	Severely affected	40	1	2	0	40	7	19	2	40	5	1	1
	4	Mildly affected	40	2	3	1	40	9	17	1	40	1	1	0
Canterbury (d)	5	Healthy	40	1	0	0	40	5	19	0	38	0	2	1
	6	Healthy	40	1	0	0	40	5	17	3	40	2	4	0

Note. No wilt was seen on site (b).

status of the ground on which they were planted. This is demonstrated by the high wilt incidence on site (c) which had previously borne a crop of severely wilted plants. The reason for the low wilt incidence on the other " disease site " (d) is obscure, but this result suggests that wilt incidence may be influenced by other factors in addition to the presence or absence of fungal inoculum in the soil.

2. *Plant vigour and crop in the second year.*

The average height and spread measurements in inches and crop in pounds per plant in the experiments conducted on sites (a) and (c) are given in Table II.

TABLE II.

Source of runners.	Site (a). " Clean " soil.			Site (c). Infected soil.		
	Height (in.).	Spread (in.).	Crop (lb.).	Height (in.).	Spread (in.).	Crop (lb.).
1	9·9	19·2	·74	8·9	18·1	·50
2	9·1	17·4	·59	8·3	16·1	·50
3	10·0	20·9	·78	8·6	16·1	·59
4	8·5	16·4	·52	8·4	16·7	·55
5	10·0	19·5	·75	8·5	17·1	·46
6	9·6	20·2	·68	8·6	17·6	·65

An analysis of the records indicated that on site (c) the plants from all six sources behaved similarly in respect of vigour and cropping. On site (a), however, the plants in categories 2 and 4 (i.e. initially from mildly affected plants) were smaller and gave less crop than did those in any of the other categories.

The differences between these plants and those in categories 1 and 3 (from severely affected parent plants) may have been due to the differences in initial size already noted. A comparison with the runners from healthy plants which also came from crowded blocks suggests, however, that some other factor was also involved which may have been the presence of *Verticillium*. As no comparable healthy plants were available from the diseased sites, however, it was not possible to determine this.

It is noteworthy that in spite of the high wilt incidence in the maiden year the plants on site (c) gave a fairly reasonable crop in the second year (about 2 tons per acre at the planting distance used—3 ft. by 2 ft.).

COMPARISON OF WILT INCIDENCE IN DE-RUNNERED AND RUNNERED PLANTS.

As already noted, during the field surveys wilt was often seen in one-year plantations on which runners were being produced. It seemed possible that plants forming runners, and thus presumably subject to greater losses of water by transpiration, might, until the runners were rooted in the soil, show more severe wilt symptoms than de-runnered plants.

A small observational trial was made in 1946 to compare the wilt incidence in de-runnered and runnered plants in two categories, viz., (*a*) taken from severely wilted parent plants at East Malling and grown on in wilt-free soil and (*b*) taken from healthy parent plants and grown on in wilt-affected soil. Healthy runners were also grown as controls on the supposedly wilt-free site and, since these showed wilt, apparently the soil here was actually slightly infected.

The plants were grown at a spacing of 3 × 3 ft., the runnered plants allowed to form their runners on the "block system" and the others regularly de-runnered.

TABLE III.

Wilt incidence in de-runnered and runnered plants on two sites.

Site.	Parent Stock.	De-runnered plants.					Runnered plants.				
		No.	W?	W	W+	Total Wilt.	No.	W?	W	W+	Total Wilt.
"Wilt-free" soil	Diseased	48	11	2	0	2	47	20	1	0	1
	Healthy	47	3	6	0	6	47	19	5	0	5
Wilt-infected soil	Healthy	31	2	11	0	11	32	9	19	3	22

As shown in Table III, 22 of the runnered plants on wilt infected soil showed definite wilt symptoms compared with 11 de-runnered plants. Although not suitable for statistical analysis, this result suggests that allowing the plants to form runners increased the severity of the wilt symptoms. Apart from the greater incidence of possible wilt on the runnered plants, the part of the experiment on the "wilt-free" soil does not show any more wilt in the runnered plants than in those that were de-runnered. Nor was there any more wilt in the plants from infected parent stock than in those from healthy stock. This result confirms that of the other trial already described, and also shows that the formation of runners by plants taken from infected parent stock does not cause them to show symptoms.

THE EFFECT OF CERTAIN PREVIOUS CROPS ON WILT INCIDENCE AND SEVERITY.

A field trial on this subject was laid down in 1947 on a plot of ground at the Research Station, 200 yds. long and 20 yds. wide, and known to be infected with *V. dahliae*. In 1945 some 75 per cent. of the plants in a Special Stock strawberry runner-bed on this site had shown such severe wilt symptoms that the bed was of no commercial value. Numerous isolations of *V. dahliae* were made from the affected plants. In 1946 the ground was cropped with peas.

In 1947 the strip of ground was divided into four blocks, each containing six plots, 160 square yards in area, thus providing for six crop treatments replicated four times. The crops used were potatoes (var. Majestic), peas, mangolds and clover ley.* The potatoes were obtained from three different sources (Scotch seed), those from each source being regarded as a different crop. This was done with the expectation that if any appreciable amount of infection was introduced from any source it would become evident by comparison with the other two. It was not possible to estimate the incidence of *Verticillium* in the tubers.

The peas, ley and mangolds were sown on 14th April and, on 14th May, the potatoes were planted at the rate of 270 tubers per plot. This was wider than normal spacing because of shortage of tubers.

The crops grew fairly well but the plots sown with peas became rather weedy,

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* 12 lb. S24 perennial ryegrass, 9 lb. English broad leaved red clover, 1 lb. English trefoil and 1 lb. Canadian Alsike clover per acre.

Chenopodium album being particularly prevalent. A few scattered potato plants showed symptoms of *Verticillium* wilt and *V. dahliae* was isolated from them. No manures were applied to the plots in 1947. The peas were harvested when ripe and the weeds on these plots were then allowed to grow freely. The mangolds and potatoes were harvested in the autumn and the whole area was ploughed and harrowed soon afterwards.

On 15th November, 252 Special Stock Huxley strawberries were planted on each plot at a spacing of 2 × 3 ft. In order to avoid edge effects, two rows of plants at the ends and sides of each plot were treated as guard rows, leaving 136 experimental plants per plot (544 per treatment).

Records of plant height and spread and of wilt incidence were made in the autumn of 1948, and are summarized in Table IV.

TABLE IV.

Average size and % wilt of strawberries grown on plots which had borne various crops in the previous year.

1947 Crop.	1948. Strawberry records.				
	Av. height (in.).	Av. spread (in.).	% severe wilt.	% moderate wilt.	% missing plants.
Potatoes A	4·8	10·6	8·5	20·4	3·3
" B	4·5	10·2	7·9	23·2	2·9
" C	4·7	10·7	8·1	20·4	1·7
Peas ..	4·7	10·8	5·5	18·0	1·8
Mangolds ..	4·8	11·1	4·8	17·3	3·1
Ley ..	5·2	12·4	2·6	13·8	5·0

A statistical analysis of the records on which this summary is based showed that wilt incidence on the plots which had grown potatoes was unrelated to the source of the tubers. It may, therefore, be concluded either that none of the tubers from any source introduced fresh infection or that they all introduced such infection equally.

If the three potato treatments are grouped, the statistical relationships of wilt incidence after each crop can be expressed as follows. Treatments which were not significantly different are bracketed together.

% Severe Wilt*	Potatoes.	Peas.	Mangolds.	Ley.
	8·4	5·6	4·9	2·7
	└───┬───	───┴───┬───	───┴───┬───	───┘
% Total Wilt*	Potatoes.	Peas.	Mangolds.	Ley.
	30·3	24·0	22·8	17·2
		└───┬───	───┴───┬───	───┘

* Calculated as a percentage of surviving plants.

The total wilt incidence on the plots where potatoes had been grown was significantly greater than on any other plot. Wilt incidence was least on the ley plots and significantly different from that on the pea and potato plots, but not from that on the mangold plots. The incidence of severe wilt is parallel to that of total wilt, although there were fewer significant differences.

The height and spread measurements show that the plants grown after ley were significantly larger than those on any other plot. There were no significant differences between the treatments in missing plants, although there were most of these on the ley plots.

The results of this experiment provide evidence that, if wilt-infected ground is cropped with potatoes in the year before strawberries are planted, wilt incidence in the strawberries will be greater than if ley, mangolds or peas are used as a prior crop. Of the four crops tested the one-year ley resulted in the lowest wilt incidence and the largest plants. Further and longer term experiments are obviously advisable to determine whether, as this experiment suggests, a clover ley will tend to reduce the danger of wilt in strawberries. Recent observations* indicate, however, that red clover may introduce or encourage the stem eelworm (*Ditylenchus dipsaci* Kühn) which is capable of causing damage to strawberry plants. It would, therefore, seem advisable in any case to exclude red clover from any swards sown in preparation for planting ground with strawberries.

TESTS OF VARIETAL SUSCEPTIBILITY TO *V. albo-atrum.*

Thomas (1932) reported that certain American varieties of strawberry showed some resistance to *Verticillium* wilt. More recently Baker and Baker (1950) have reported significant differences between varieties in their reaction to wilt infection and have suggested types of plot layout suitable for further study of this matter.

In the present investigation the reaction of a number of strawberry varieties to infection with *V. albo-atrum* was studied in 1946 and 1947. No Californian varieties were available, but a wide range of other varieties was used from the collection maintained at the Research Station. Some were tested in both years and others in one year only, according to the availability of runners.

In both trials the strawberries were planted in triplicate plots arranged in randomized blocks to avoid possible soil effects. The number of plants used per variety was 27 in 1946 and 30 in 1947. In 1946 the site used was a plot of ground on a heavy clay-loam soil which had been artificially infected in 1945, by incorporating with the soil stems from hop plants suffering from severe (progressive) *Verticillium* wilt. This site had been used in 1945 for a wilt resistance trial of hops, most of which had shown severe symptoms. No further inoculum was added in 1946. The 1947 experiment was carried out at the Research Station on a medium sandy loam soil. The plants were infected by putting 10-12 gm. of chopped, dry, naturally infected hop stem in each of the planting holes. This infected hop stem was similar to that used in 1945 and had been collected from hop plants infected with progressive wilt in 1946.

As the plants grew for one year only in each of the trials, no precautions were taken to isolate the virus-tolerant and expressive varieties and there was little evidence of variation in the plants due to virus infection. There were, however, very considerable differences in the vigour of the varieties, and it was difficult to estimate accurately the wilt status of those that grew poorly. In 1947 many plants died, apparently from drought, making the numbers of each variety very uneven.

Final records were made in October, 1946, and in September, 1947. Table V summarizes these records, giving the numbers of plants recorded of each variety and the percentage showing wilt symptoms.

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* By R. S. Pitcher, East Malling Research Station. (Unpublished.)

TABLE V.

Test of susceptibility of strawberry varieties to wilt induced by V. albo-atrum.

Variety.	1946 Trial.		1947 Trial.	
	No. recorded.	% wilt.	No. recorded.	% wilt.
Oberschlesien	27	48	18	89
Mme. Lefebvre	27	44	23	100
Tardive de Leopold	26	54	15	100
Huxley's Giant	26	46	29	97
Royal Sovereign	26	15	27	97
Paxton	15	20	22	87
Perle de Prague	20	30	17	94
Bradley Cross	24	21	23	96
King George	21	4	22	91
Early Cambridge	22	54	20	95
Auchincruive Climax	26	23	24	92
Auchincruive 10	25	16	28	90
Auchincruive 1	22	31	—	—
Auchincruive 4	25	40	—	—
L.R. 19	22	27	—	—
The Duke	23	17	—	—
Aberdeen	27	26	—	—
Georg Soltwedl	—	—	20	100
Cambridge 55	—	—	22	91
112	—	—	20	100
151	—	—	19	100
276	—	—	17	94
504	—	—	26	100
660	—	—	24	100

As shown in the table, almost all the plants of every variety tested in 1947 showed wilt symptoms. It is thus clear that none was resistant to infection with *V. albo-atrum* under the apparently severe condition of this experiment. In 1946, however, differences were shown between the varieties, both in wilt-incidence and in vigour. Those that appeared most vigorous and on which the effect of wilt was least marked were King George, Royal Sovereign and Auchincruive 10. The most severely affected varieties were Early Cambridge, Oberschlesien, Huxley, Madame Lefebvre and Tardive de Leopold. It is thus possible that the varieties tested may differ in resistance to wilt but that under conditions favouring the disease these differences may not be apparent.

The results of these experiments do not indicate any possibility of control by means of resistant varieties. It should be noted, however, that *V. albo-atrum* was used in both trials and the reaction of the varieties to *V. dahliae* is unknown. The trials have shown, however, that very variable results are likely in trials of this sort which may thus only be of value for the detection of outstanding resistance.

DISCUSSION.

The observations and experiments described do not indicate any means for the control of the disease. It seems likely that the pathogens may be found in many localities and on many types of soil. It also appears that, on some soils and in certain seasons at least, runners from severely affected plantations may produce apparently normal plants, giving good crops both of runners and fruit. This suggests that the infectivity of the soil is the prime factor in determining wilt incidence in a new plantation. In this connection, the recent studies of Wilhelm (1950) are important. He tested samples of soil from 20 sites in California by growing tomatoes in them and demonstrated the presence of *Verticillium albo-atrum* (*V. dahliae* ?) at varying depths down to 3 ft. He concluded that in some cases, *Verticillium* may persist in the soil for long periods. He also suggests that the infection index of a soil is related directly to inoculum potential.

The experiment on the effect of previous crops and the evidence from field observations support the view of Thomas that cropping with other wilt susceptible plants may increase wilt incidence in strawberries subsequently planted on the ground. It is by no means an invariable rule, however, that ground previously cropped with, say, potatoes will induce wilt in strawberries or, conversely, that ground in which no susceptible crop has been grown for some years will be free from the disease. In view of the evidence already available, however, it seems advisable to avoid ground that has recently borne a crop of potatoes if, for instance, a valuable special stock nursery is being planned.

As already stated, *Verticillium* wilt is of most importance in runner beds. Apart from the reduction it causes in the number of runners produced, the disease has an important secondary effect, in simulating or masking the symptoms of the virus disease Yellow Edge, thus making the diagnosis of both these diseases very difficult (Prentice, King and Harris, 1946). It has, therefore, sometimes been necessary, during inspection under the Ministry of Agriculture Certification Scheme, to reject runner beds in which there was a high wilt incidence, owing to the impossibility of assessing the degree of infection with virus diseases.

Thomas suggests that *Verticillium* wilt of strawberries may eventually be controlled by the use of resistant varieties. No British variety possessing marked resistance has been found either in the experiments or in field surveys, and the possibility of control by this means seems rather remote.

SUMMARY.

An account is given of observations and experiments on *Verticillium* wilt of the strawberry in Britain. The symptoms are described and notes given on the pathogens, *V. albo-atrum* and *V. dahliae*. The disease causes the greatest losses in the first year of growth of the plants and is thus of most importance in runner beds. It is particularly prevalent in the Wisbech area, but has also been seen in many other localities. Experiments on four different sites indicate that runners taken from severely affected plantations may produce apparently normal plants, and it is suggested that the prime factor in determining wilt incidence is the infectivity of the soil. Some evidence has been obtained that this is affected by prior cropping with potatoes. None of the common British varieties was found to show resistance to wilt caused by *V. albo-atrum* and no immediate control measures can be recommended.

ACKNOWLEDGMENTS.

The writers wish to acknowledge their indebtedness to the members of the Statistics Section of the Research Station for their help in the analysis of the experimental results ; also to the officers of the National Agricultural Advisory Service who have collaborated in the collection of field data.

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PLATE I.

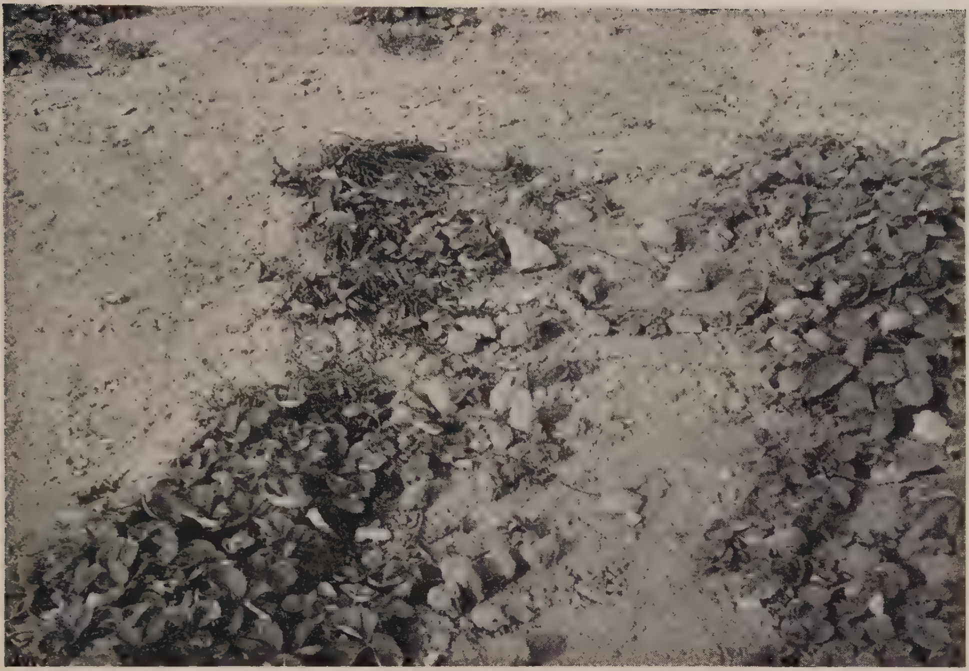

Fig. 1.
Block of strawberry runners showing one plant severely affected by *Verticillium* wilt.

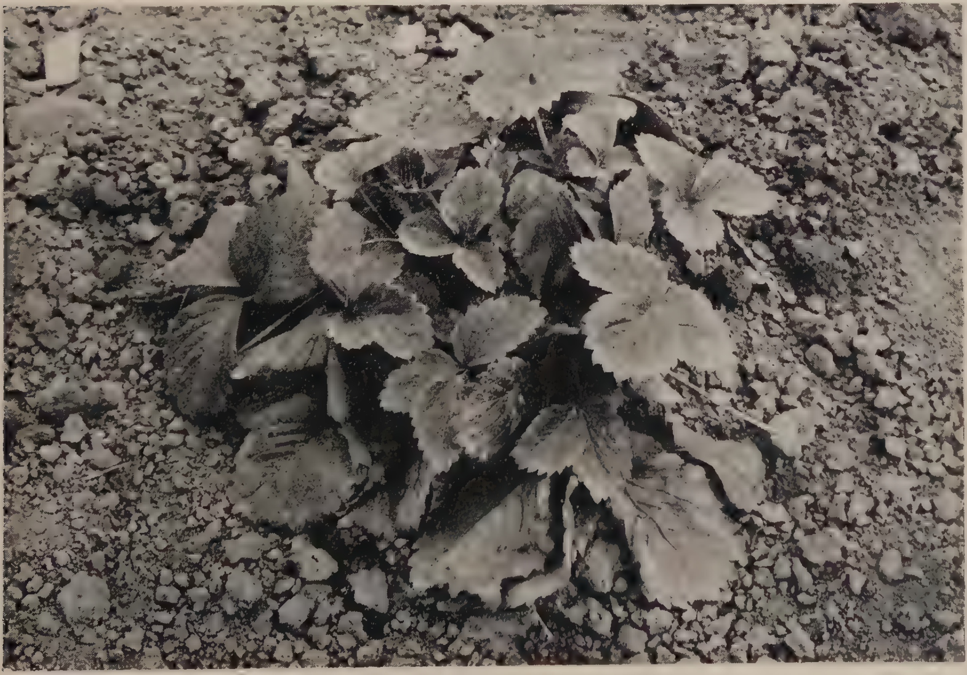

Fig. 2.
Strawberry plant (var. Huxley) showing mild wilt symptoms.

PLATE II.

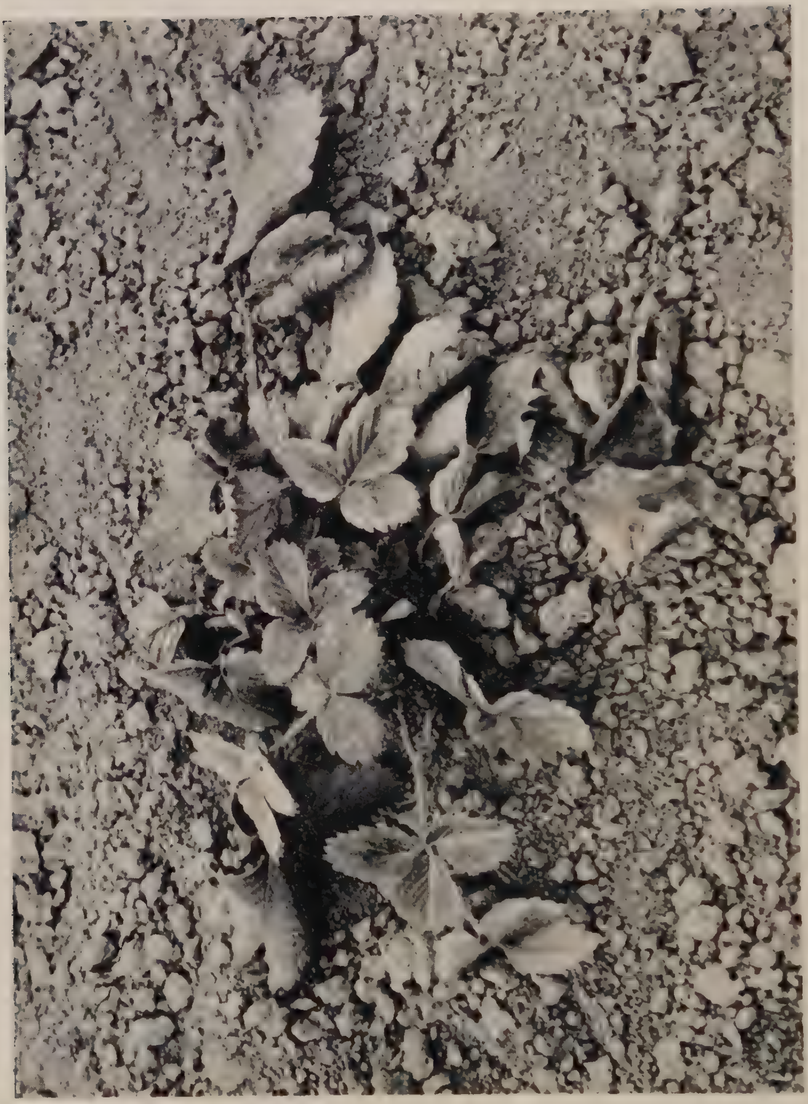

Fig. 3

Strawberry plant (var. Huxley) showing severe wilt symptoms

STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES

III. THE PRODUCTION OF PARTHENOCARPIC POMACEOUS FRUITS BY CHEMICAL TREATMENT

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SYNOPSIS.

An account is given of the work carried out during a series of investigations into the production of seedless pomaceous fruits following applications of synthetic growth-regulating substances.

A wide range of compounds was studied and mature seedless fruits were obtained using one substance on certain varieties of pear. Preliminary experiments have indicated that another compound can be effective for *Bramley's Seedling* apples.

INTRODUCTION.

ALTHOUGH much work has been done on the production of parthenocarpic fruits by the use of plant growth-regulating substances (see e.g. 1, 12) the attempts to induce parthenocarpy in pomaceous fruits have so far met with only limited success.

Small sets of parthenocarpic pears have been obtained by Sereisky (6), who removed ovules from small fruits of *Pyrus communis Caucases* and replaced them with a lanolin paste containing phenylacetic acid. He also applied lanolin solutions of phenylacetic acid and indole-3-acetic acid to the cut ends of the styles of flowers which had previously had stamens and stigmas removed, and obtained sets of mature pears varying from 23 to 30 per cent.

Van Stuivenberg (8) treated frosted pear blossoms, var. *Précoce de Trevoux* with lanolin paste containing a mixture of indole-3-acetic acid and potassium naphthylacetate, and a 42·1 per cent. set of mature parthenocarpic fruit was harvested. Swarbrick (10) induced the initial set and development of unemasculated blossoms of *Pitmaston Duchess* by the application of aqueous sprays of 2-naphthoxyacetic, 2 : 4-dichlorophenoxyacetic and 2 : 6-dichlorophenoxyacetic acids.* None of the fruitlets, however, attained more than a quarter of full size.

Several attempts to produce parthenocarpy in apples by treatment with synthetic growth-regulating substances have been recorded. Varrelman (11) sprayed emasculated and protected blossoms of several varieties of apple, including *Spencer Navel* which is normally parthenocarpic, with indole-3-butyric acid and 1-naphthylacetic acid, but failed to induce fruit-set. Greene (2) found that applications, one month after flowering, of 1-naphthylacetic, indole-3-acetic, indole-3-propionic and indole-3-butyric acids, in the form of lanolin pastes, sprays and branch injections, caused a retardation in the development of the fruitlets. Swarbrick (9) sprayed swelling, frosted fruitlets of *Cox's Orange Pippin* and *Miller's Seedling* with a mixture of growth substances containing indole-3-butyric acid (5 p.p.m.),

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* The sample of 2 : 6 acid used by Swarbrick has since been shown (5) to have contained 2 : 4-dichlorophenoxyacetic acid, which was probably responsible for the growth-regulating activity shown.

1-naphthylacetic acid (5 p.p.m.), 2 : 4-dichlorophenoxyacetic acid (5 p.p.m.) and 2-naphthoxyacetic acid (60 p.p.m.). Most of the unsprayed blossoms abscissed on both varieties but, on *Miller's Seedling* only, the sprayed fruitlets continued to grow, many remaining on the tree until maturity and some attaining a diameter of 1½-2 in. It should be noted that in this trial, blossoms had been normally fertilized and were swelling rapidly before frost killed the embryos and blackened the cores of all fruits. It would appear, therefore, that parthenocarpy was not initially induced by growth substance treatment.

Swarbrick (10) sprayed mature trees, just after petal fall, with various concentrations of 2 : 4-dichlorophenoxyacetic acid and obtained a complete initial set of *Bramley's Seedling* apple, and a partial set of *Edward VII*, though in all cases the fruitlets made only little growth. No effect was obtained on *Allington Pippin* or *Cox's Orange Pippin.*

EXPERIMENTS WITH PEARS.

Preliminary experiments using a range of synthetic growth substances were carried out by one of us (R.L.W.) in collaboration with Dr. I. W. Selman during 1947. These gave an indication that a single spray of α-(2-naphthoxy)propionic acid at 100 p.p.m., or one spray of a mixture containing 2 : 4-dichlorophenoxyacetic acid (5 p.p.m.), 1-naphthylacetic acid (5 p.p.m.), 2-naphthoxyacetic acid (50 p.p.m.) and α-(2-naphthoxy)propionic acid (50 p.p.m.) would induce the initial swelling of emasculated blossoms of the pear varieties *Pitmaston Duchess* and *Beurré Superfin*. Growth of these fruitlets ceased, however, after some three weeks.

It was concluded from this, that the original stimulus to growth which the single chemical treatment had clearly supplied, was insufficient to promote continued development. Accordingly, the fruitlets which had shown this initial swelling and had ceased to grow, were sprayed for a second time with the same solution as before. No further development resulted and the fruitlets soon abscissed.

1948 Experiments.

A full scale experiment was carried out on two varieties of pear, *Pitmaston Duchess* (triploid), and *Dr. Jules Guyot* (diploid). The following methods were employed for supplying a continuous source of growth substance to fruitlets whose development had been initiated from emasculated blossoms by a single spray application of α-(2-naphthoxy)propionic acid at 100 p.p.m.

(*a*) Spraying the fruitlets at three-day intervals with an aqueous solution of the sodium salt of the growth substance, incorporating a wetting agent of the sodium secondary alcohol sulphate type.

(*b*) Applying the compound as the free acid in lanolin solution to the young fruitlets, thereby providing a supply of growth substance for a considerable period.

(*c*) Injection of an aqueous solution of the sodium salt into the ovary using a micro-hypodermic syringe.

(*d*) Supplying the young fruitlets with an aqueous solution of the sodium salt of the compound by immersing the mid-rib of the subtending leaf in a small tube, containing the solution, wired on to the truss. (This method was worked out by Roach and was developed from other methods designed by him (4) for mineral nutrition studies.)

Although α-(2-naphthoxy)propionic acid was used in all these four methods a small experiment was carried out involving the respraying of developing fruitlets with solutions of either indole-3-butyric acid or 1-naphthylacetic acid. It was considered that the initial stimulation of development supplied by α-(2-naphthoxy)-propionic acid might well be continued by the subsequent application of a substance known to be active for cell extension (indole-3-butyric acid), or by a substance which might prevent the formation of an abscission layer at the base of the fruitlet (1-naphthylacetic acid). No such effect was noted in these experiments, neither treatment resulting in continued growth.

In the main series of experiments, using α-(2-naphthoxy)propionic acid, the fruit injection and leaf feeding techniques were not successful, and although the lanolin treatment did yield 25 per cent. of mature fruit on *Dr. Jules Guyot*, by far the best response was obtained from the spray treatments. Only these latter results are described.

Some 750 blossoms were emasculated in the white-bud stage, by removing the petals, stamens and stigmas with a sharp scalpel. Although it is known that the position of a blossom on a truss may influence its chance of natural setting (7), in the present experiments, selection of blossoms for emasculation was determined by stage of development only. All trusses were reduced to five blossoms. The emasculated blossoms were immediately sprayed to run-off using a hand atomizer. Treatments and concentrations of spray used were as follows, eight trusses each carrying five emasculated blossoms being used per treatment.

1. Sprayed once with α-(2-naphthoxy)propionic acid at 100 p.p.m. and wetting agent.
2. Sprayed once with α-(2-naphthoxy)propionic acid at 250 p.p.m. and wetting agent.
3. Sprayed once with α-(2-naphthoxy)propionic acid at 1,000 p.p.m. and wetting agent.
4. Sprayed every three days with α-(2-naphthoxy)propionic acid at 100 p.p.m. and wetting agent.
5. Sprayed once with water and wetting agent.

In addition, 16 normal trusses (80 blossoms) were labelled for observation and recording as unsprayed controls.

The most effective treatments on *Pitmaston Duchess* were the single spray at 250 p.p.m. and the three-day respray at 100 p.p.m., although spraying with α-(2-naphthoxy)propionic acid at any concentration induced rapid initial growth (Fig. 1). After 31 days the largest fruits on the trees were those arising from the three-day respray treatment, though by the end of June there was no difference in size between treated and normally fertilized fruits.

The *Dr. Jules Guyot* variety was found to be more sensitive to treatment with α-(2-naphthoxy)propionic acid than *Pitmaston Duchess*. Thus, from the point of view of the number of pears that reached maturity and were harvested, the best results on *Dr. Jules Guyot* were obtained with the single spray of α-(2-naphthoxy)-propionic acid at 100 p.p.m. However, abscission of fruitlets was delayed much longer by the single spray at 250 p.p.m. and by the resprays at 100 p.p.m. and further, both these treatments showed the highest percentage of half-grown fruits. There was a high rate of abscission of these fruitlets during the period coinciding with

"June-drop" and within 20 days many had fallen from the trusses which had received these treatments. Trusses which were repeatedly resprayed eventually shed all fruit, a result which might well be attributed to over-treatment.

The final percentages of parthenocarpic fruit harvested in both varieties are shown in Table I.

TABLE I.

Percentage parthenocarpy induced in Pitmaston Duchess and Dr. Jules Guyot by treatment with α-(2-naphthoxy)propionic acid.
(1948 Experiments.)

Treatment.	*Pitmaston Duchess.*	*Dr. Jules Guyot.*
1. Single spray (100 p.p.m.)	10	31
2. " " (250 p.p.m.)	25	13
3. " " (1,000 p.p.m.)	13	0
4. Sprayed every three days (100 p.p.m.)	38	0
5. Sprayed once (water and wetting agent only) . .	13	13
Percentage of unemasculated blossoms which grew to give mature fruit	19	38

On *Pitmaston Duchess* 19 per cent. of the normally fertilized blossoms developed into mature pears. Thirty-eight per cent. developed following the three-day respray treatment at 100 p.p.m. and 25 per cent. with the single spray at 250 p.p.m. All treated fruits, including those sprayed once with water and wetting agent were completely parthenocarpic, containing only tiny flattened pips without embryos. Examination of some 150 normally fertilized fruits, however, showed that 45 per cent. of these mature pears were also parthenocarpic ; there was an average pip content of 0·7 per pear. Since 19 per cent. of the untreated blossoms grew to maturity it would appear that some 9 per cent. of the original blossom on these trees of *Pitmaston Duchess* was capable of natural development into parthenocarpic fruit during the year 1948.

This 9 per cent. might well account for the yields of mature parthenocarpic fruit obtained from the 100 p.p.m. and 1,000 p.p.m. treatments and from the "water-and-wetter" spraying, but it is unlikely that the yields of 25 and 38 per cent. obtained with the single spray at 250 p.p.m. and the resprays at 100 p.p.m. resulted from natural parthenocarpy. In these two cases, therefore, it would appear that the α-(2-naphthoxy)propionic acid treatments had stimulated parthenocarpic development to maturity (3).

On *Dr. Jules Guyot* 38 per cent. of the original blossom gave rise to mature fruits and all contained from 1 to 9 pips (average 5·8) with fully developed embryos. There appeared to be no natural parthenocarpy in this variety. The single spray of α-(2-naphthoxy)propionic acid at 100 p.p.m. caused 31 per cent. of the original blossom to reach maturity, and a value of 13 per cent. was obtained with the 250 p.p.m. treatment. All these fruits were completely parthenocarpic, with tiny flattened seeds containing a shrivelled embryo-sac (Fig. 2).

It would appear, therefore, that the production of parthenocarpic fruit following the "water-and-wetter" spray on *Dr. Jules Guyot* cannot be explained by any inherent capacity for parthenocarpy. Since all the treatments were carried out on the same tree, however, it is possible that, even though care was taken when respraying, some spray drift, or translocation effects, may have stimulated the development of these blossoms. The possibility, though, could not be overlooked that the emasculation process or treatment with water containing wetting agent, might have induced parthenocarpic development in these controls. These possibilities were therefore investigated in the following year's work.

1949 Experiments.

These experiments were designed to determine (*a*) whether emasculation itself is capable of inducing parthenocarpic development, perhaps by a wounding stimulus, (*b*) whether a single spray or spraying every three days with water and wetting agent might induce parthenocarpic development, (*c*) the number of sprays of α-(2-naphthoxy)propionic acid at 100 p.p.m. necessary to give optimum set and development of fruitlets in each variety. The programme involved the emasculation of some 1,000 blossoms on *Pitmaston Duchess* and *Dr. Jules Guyot*, 5 trusses each carrying 5 blossoms being used for each of the 14 treatments described in Table II. In addition, on each variety 20 trusses (100 emasculated blossoms) received water and wetter treatment only and 10 trusses (50 unemasculated blossoms) were labelled for recording as unsprayed controls.

Table II shows the percentage of attached fruitlets on each variety from 19 days after spraying until harvest.

On *Pitmaston Duchess* nearly 100 per cent. of blossoms sprayed with growth substance were still growing after one month. A large number of the emasculated blossoms which had received water and wetting agent or which had been left unsprayed had already abscissed by this time. The final figures listed in Table II represent mature parthenocarpic pears harvested. Five resprays of α-(2-naphthoxy)-propionic acid gave the highest yield of mature fruit ; indeed the yield of fruit harvested from the 5-respray treatment was considerably higher in this variety than that obtained from normally fertilized blossoms.

On *Dr. Jules Guyot* after one month, there was a 90-100 per cent. set of blossom following treatment with α-(2-naphthoxy)propionic acid, while most of the emasculated blossom left unsprayed or which had been sprayed with water and wetting agent had abscissed. After seven weeks the only fruitlets remaining on the trees were those arising from chemical treatment or from normal fertilization. "June-drop" took a somewhat heavier toll of the parthenocarpic fruits in this variety than in *Pitmaston Duchess*, though again the highest yield of mature fruits was obtained from the 5-respray treatment (Table II).

Mature fruits from both varieties which had developed from emasculated blossoms were sectioned and all were found to be completely parthenocarpic. Some 200 normally fertilized fruits of *Pitmaston Duchess* were also examined and 59 per cent. of these were found to possess small loculi containing undeveloped ovules. This represents the percentage of natural parthenocarpy occurring in these trees of *Pitmaston Duchess* during 1949. The average pip number of all the fruits examined was 0·6. Of 100 normally fertilized fruits examined from *Dr. Jules Guyot*, none was parthenocarpic and the average pip number was found to be 6·1.

1950 Experiments.

A limited experiment was carried out, using the five-respray treatment of α-(2-naphthoxy)propionic acid at 100 p.p.m. on emasculated blossoms of *Pitmaston*

TABLE II.

Percentage attached fruitlets on Pitmaston Duchess and Dr. Jules Guyot following treatment of emasculated blossoms.*
(1949 Experiments.)

DAYS AFTER SPRAYING.	EMASC. CONTROLS.	WATER AND WETTER SERIES.							COMPOUND SERIES.							NORMALLY FERTILIZED.
		No. of times sprayed.							No. of times sprayed.							
		1	2	5	7	9	11	13	1	2	5	7	9	11	13	
19	100 *99*	100 *100*	92 *96*	100 *100*	100 *100*	100 *96*	100 *100*	100 *100*	100 *96*	100 *100*	100 *96*	100 *96*	100 *100*	100 *100*	100 *100*	100 *100*
31	39 *23*	48 *0*	16 *8*	8 *0*	8 *0*	8 *0*	0 *0*	8 *12*	60 *92*	100 *96*	100 *92*	100 *96*	100 *92*	100 *100*	100 *91*	60 *96*
40	20 *2*	40 *0*	16 *0*	8 *0*	4 *0*	8 *0*	0 *0*	4 *0*	44 *52*	88 *56*	100 *92*	96 *80*	96 *92*	96 *96*	92 *66*	42 *68*
52	11 *0*	20 *0*	12 *0*	8 *0*	4 *0*	8 *0*	0 *0*	4 *0*	36 *16*	60 *24*	72 *36*	68 *64*	60 *72*	76 *64*	70 *40*	28 *52*
60	5 *0*	8 *0*	0 *0*	0 *0*	0 *0*	0 *0*	0 *0*	0 *0*	28 *8*	48 *12*	44 *20*	28 *8*	36 *0*	44 *8*	17 *8*	24 *28*
90	4 *0*	4 *0*	0 *0*	0 *0*	0 *0*	0 *0*	0 *0*	0 *0*	28 *4*	36 *4*	40 *12*	16 *0*	16 *0*	32 *0*	7 *0*	16 *24*
110	4 *0*	4 *0*	0 *0*	0 *0*	0 *0*	0 *0*	0 *0*	0 *0*	28 *4*	36 *4*	40 *12*	16 *0*	16 *0*	32 *0*	7 *0*	16 *24*

* The figures for this variety are given in italics.

Duchess and *Fertility*. Eighteen per cent. of the treated, emasculated blossoms developed to maturity on *Pitmaston Duchess* and 20 per cent. of the sprayed blossoms developed to maturity on *Fertility* (Fig. 3).

As in previous years a few emasculated unsprayed controls developed to give mature parthenocarpic fruits on *Pitmaston Duchess*. In this variety there was again a high percentage of natural parthenocarpy in normal fruits. None of the control emasculated blossoms developed on *Fertility*, and the normally fertilized fruits examined contained many fully grown seeds (Fig. 3).

EXPERIMENTS WITH APPLES.

A selected range of the synthetic growth-regulating substances prepared during the course of the present investigations was sprayed on to emasculated blossoms of apples.

Unopened blossoms, in the pink-bud stage, were emasculated with a sharp scalpel, by removing stamens, stigmas and petals. Within an hour of emasculation, aqueous solutions containing the sodium salts of the respective compounds together with wetting agent, were applied, using a small hand atomizer. In all cases, the trusses were reduced to four blossoms.

Treated trusses which showed any signs of swelling were resprayed, using the same compound. *Bramley's Seedling* was used in each year's experiments, but certain compounds were also sprayed on to emasculated blossoms of *Edward VII* and *Monarch*. The number of blossoms which abscissed during the experiment was recorded at intervals.

1948 Experiments.

Solutions of each of the following compounds with wetting agent were sprayed once on to emasculated blossoms of *Bramley's Seedling* :

2-chlorophenoxyacetic acid (200 p.p.m.),
α-(2-chlorophenoxy)propionic acid (50 p.p.m.),
4-chlorophenoxyacetic acid (50 p.p.m.),
α-(4-chlorophenoxy)propionic acid (50 p.p.m.),
β-(2-naphthoxy)propionic acid (250 p.p.m.),
α-(2-naphthoxy)propionic acid (100 p.p.m.),
1-chloro-2-naphthoxyacetic acid (200 p.p.m.),
β-(1-chloro-2-naphthoxy)propionic acid (250 p.p.m.),
α-(1-chloro-2-naphthoxy)propionic acid (200 p.p.m.),
4-chloro-3 : 5-dimethylphenoxyacetic acid (50 p.p.m.),
2-thionaphthoxyacetic acid (200 p.p.m.).

All emasculated control blossoms which were sprayed with a solution containing water and wetting agent, abscissed within 21 days of treatment. The only compounds which caused any significant delay in abscission were α-(2-chlorophenoxy)-propionic, α-(4-chlorophenoxy)propionic, α-(2-naphthoxy)propionic and 4-chlorophenoxyacetic acids. Of these, the first two compounds induced a slight initial swelling of the fruitlets in the first 21 days following spraying. These trusses were resprayed on the 23rd day, but no further increase in size took place, and complete abscission occurred within 6-7 weeks of the initial treatment.

Although blossoms sprayed with α-(2-naphthoxy)propionic acid remained on the tree longer than emasculated controls, no increase in size took place. 4-chlorophenoxyacetic acid treatment caused blossoms to remain firmly attached to the tree until the end of the season when they still appeared as fresh and green as when originally emasculated. This substance would seem to deserve further investigation in relation to the prevention of " June-drop " and " pre-harvest-drop " of apples.

1949 Experiments.

Solutions of the following compounds and wetting agent were sprayed once on to emasculated blossoms of *Bramley's Seedling* and *Edward VII*, the experiments being carried out as in the previous year :

α-phenoxypropionic acid (100 p.p.m.),
α-phenoxy-*n*-butyric acid (100 p.p.m.),
α-(2 : 4-dichlorophenoxy)-*n*-butyric acid (100 p.p.m.),
1-naphthoxyacetic acid (250 p.p.m.),
α-(1-naphthoxy)propionic acid (100 p.p.m.),
α-(1-naphthoxy)-*n*-butyric acid (250 p.p.m.),
2-naphthoxyacetic acid (50 p.p.m.),
α-(2-naphthoxy)propionic acid (50 p.p.m.),
α-(2-naphthoxy)phenylacetic acid (20 p.p.m. and 10 p.p.m.),
3 : 5-dinitrobenzoic acid (500 p.p.m.).

Most of the blossoms sprayed with a compound or with water and wetting agent abscissed within 20 days of treatment and showed no signs of swelling. Blossoms on both varieties which had received sprays of α-phenoxypropionic acid or α-(2 : 4-dichlorophenoxy)-*n*-butyric acid, however, showed a definite initial increase in size, the receptacles assuming a rounded shape. These fruitlets were resprayed, 25 days from the start of the experiment, but growth had by this time almost ceased, and all fruitlets abscissed during the following two weeks.

1950 Experiments.

In these experiments, the most promising compounds of the two previous years, and several other synthetic growth substances, were sprayed on to emasculated blossoms of *Bramley's Seedling* and *Monarch*. Spraying was repeated five times in all, with intervals of three days. The following compounds were used, made up at 100 p.p.m. with wetting agent also present :

α-phenoxypropionic acid,
α-(2-chlorophenoxy)propionic acid,
α-(2-chlorophenoxy)-*n*-butyric acid,
α-(2 : 4-dichlorophenoxy)propionic acid,
α-(2 : 4-dichlorophenoxy)-*n*-butyric acid,
2-methyl-4-chlorophenoxyacetic acid,
α-(2-methyl-4-chlorophenoxy)propionic acid,
α-(2-methyl-4-chlorophenoxy)-*n*-butyric acid,
2 : 4 : 5-trichlorophenoxyacetic acid,
α-(2 : 4 : 5-trichlorophenoxy)propionic acid,
α-(2 : 4 : 5-trichlorophenoxy)-*n*-butyric acid,
1 : 2 : 3 : 4-tetrahydro-1-naphthoic acid.

All treatments on both varieties with the exception of the 1 : 2 : 3 : 4-tetrahydronaphthoic acid spray caused blossoms to remain on the trees longer than the emasculated control blossoms which had received water and wetting agent. 2-methyl-4-chlorophenoxy and 2 : 4 : 5-trichlorophenoxy acetic and propionic acids were very active in this respect, though no growth occurred. After one month damage was apparent with these four treatments and in many cases the whole spur was eventually killed, with the undeveloped blossoms still attached.

On *Bramley's Seedling* α-(2 : 4-dichlorophenoxy)-*n*-butyric acid induced an initial swelling of the treated blossoms, the fruitlets assuming a rounded shape. The size of these fruitlets did not, however, increase beyond 0·5 cm. diameter, and most of them abscissed after 4½ weeks. α-phenoxypropionic acid induced a greater initial swelling, and all but one of the twenty-four treated blossoms remained on the tree until "June-drop" when the diameter of the fruitlets was 0·6-1·2 cm. At this stage many of the fruitlets abscissed. At the beginning of July, eight fruits were still attached to the tree ; four were small and had stopped growing, while the other fruits were of diameter 1·0, 2·0, 2·3 and 2·6 cm. respectively. These four fruits reached maturity and were found to be completely parthenocarpic.

Although the harvested fruits were smaller in size than control fruits, as far as the writers are aware this is the first recorded case of parthenocarpic development of mature apples following application of a spray of a synthetic growth-regulating substance to the emasculated blossom.

On *Monarch*, both α-phenoxypropionic and α-(2 : 4-dichlorophenoxy)-*n*-butyric acids caused some initial swellings and rounding of the fruitlets, but growth ceased after four weeks and all the blossoms fell during the "June-drop" period.

DISCUSSION.

It would appear from these results that the formation of parthenocarpic pomaceous fruits by treatment with growth-regulating substances, is a highly specific reaction. In the tomato, many compounds are known that will stimulate the formation of parthenocarpic fruit following the application of a single spray to the blossom. In the case of pomaceous fruits, however, most of these active growth substances when applied to emasculated blossoms, fail to induce a response and abscission occurs within a few days of treatment. In the present work, particularly on apple, some of the compounds employed did appear to delay the abscission of blossom, 4-chlorophenoxyacetic acid being notable in this respect. It is clear, however, that such an effect of a compound on abscission is not necessarily associated with capacity to induce fruitlet development.

One of the compounds investigated, namely α-(2-naphthoxy)propionic acid, has been shown to be highly active in stimulating the initial development of parthenocarpic fruitlets on *Pitmaston Duchess*, *Dr. Jules Guyot* and *Fertility* pears. On these varieties an almost complete initial set of the emasculated blossom was obtained and a satisfactory yield was harvested of these fruitlets at maturity.

The variety *Pitmaston Duchess* is capable of parthenocarpic development under natural conditions, however, and this may also be the case with *Dr. Jules Guyot* although we have obtained no evidence to this effect on this variety in our experiments. The possibility that the parthenocarpic development in these varieties, following application of α-(2-naphthoxy)propionic acid sprays, may have been due to an action in preventing abscission, has not been separately investigated.

On apples var. *Bramley's Seedling*, *Edward VII* and *Monarch*, α-phenoxypropionic and α-(2 : 4-dichlorophenoxy)-*n*-butyric acids were the only compounds which induced any marked swelling of the emasculated blossoms, and of these, only α-phenoxypropionic acid had the capacity to induce continued growth. Mature parthenocarpic fruits were harvested from *Bramley's Seedling* following repeated spray applications of this compound at 100 p.p.m. (Fig. 4).

The present results indicate that one application of a substance active in inducing parthenocarpic development of the apple or the pear will not normally supply the stimulus for growth to maturity. It is apparently necessary to spray several times, at short intervals to ensure continued growth. Experiments with certain pear varieties have indicated that five sprays of α-(2-naphthoxy)propionic acid at 100 p.p.m. applied at three-day intervals from the time of emasculation can induce a satisfactory yield of mature fruit.

Experiments with apples were carried out on a smaller scale but similar results were obtained. Thus application of a single spray containing α-phenoxypropionic acid to emasculated blossoms of *Bramley's Seedling* induced only slight initial development which ceased after some three to four weeks, whereas in a later experiment, five sprays at three-day intervals provided for continued development.

All these findings stand in marked contrast to the results obtained following the use of tomato setting sprays where one spray application is sufficient to stimulate growth to maturity. For the production of parthenocarpic pomaceous fruit by chemical treatment it would appear that an adequate supply of the growth-regulating substance must be made available, especially during the early stages of development. It is of interest that α-(2-naphthoxy)propionic acid which is effective in stimulating parthenocarpic development in certain varieties of pear, should fail to have induced a response with any of the apple varieties investigated. The results well illustrate the specific activity possessed by individual compounds towards different species of pomaceous fruit and the variation which can be shown between different varieties. These considerations justify the examination of a wide range of selected growth substances in these and all similar investigations.

SUMMARY.

Attempts to produce parthenocarpy in emasculated blossoms of pomaceous fruits by the application of synthetic growth-regulating compounds are described. These experiments which have extended over four years have shown that some five applications of a spray of α-(2-naphthoxy)propionic acid at 100 p.p.m. can be effective in producing mature fruits in the pear varieties *Pitmaston Duchess*, *Dr. Jules Guyot* and *Fertility*. This compound was found to be ineffective when applied to emasculated blossoms of apple varieties *Bramley's Seedling*, *Edward VII* and *Monarch*. Of some thirty other synthetic growth substances investigated, only one was found to possess the capacity to stimulate fruitlet development. This substance, α-phenoxypropionic acid, applied as a spray containing 100 p.p.m. to emasculated blossoms of the apple varieties listed above, caused some initial development. In the case of *Bramley's Seedling* only, mature fruits were obtained following several

PLATE I.

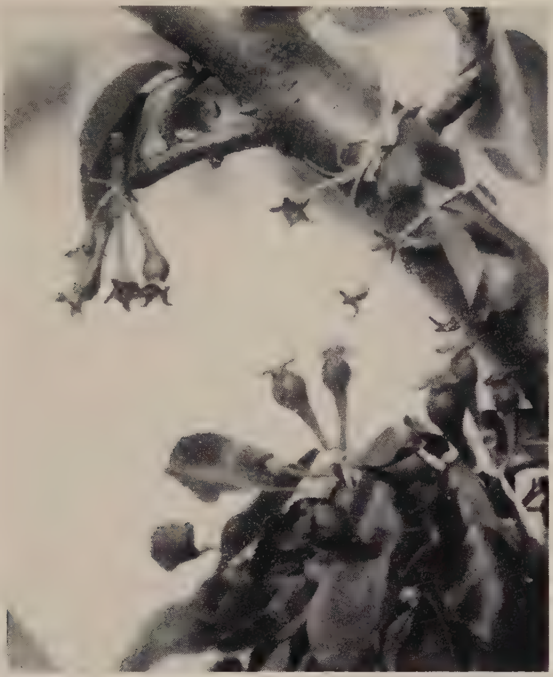

FIG. 1.

Top left : Normally fertilized fruitlets.
Top right : Control emasculated blossoms sprayed with water and wetting agent only.
Bottom : Fruitlets from emasculated blossoms sprayed at three-day intervals with α-(2-naphthoxy)propionic acid at 100 p.p.m.

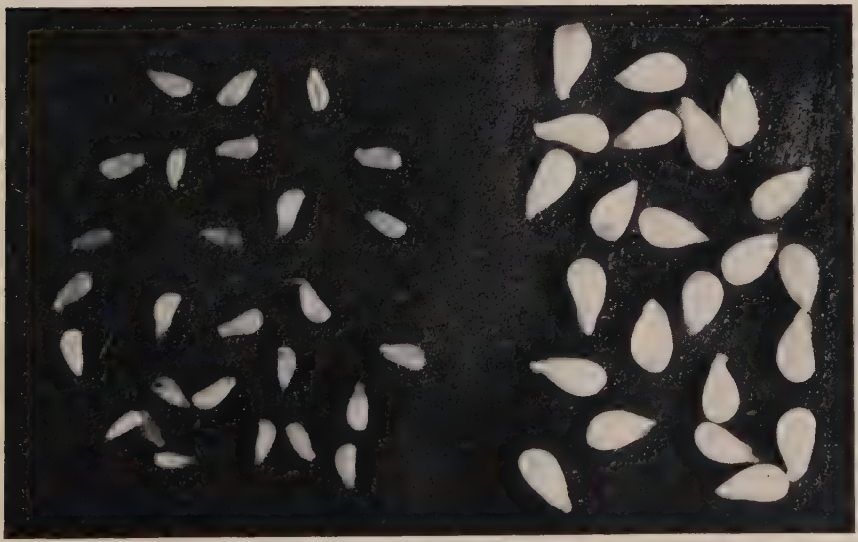

FIG. 2.

Left : Small undeveloped seeds from parthenocarpic pears produced by repeated spraying with α-(2-naphthoxy)propionic acid at 100 p.p.m.
Right : Seeds from normal pears (var. *Dr. Jules Guyot*).

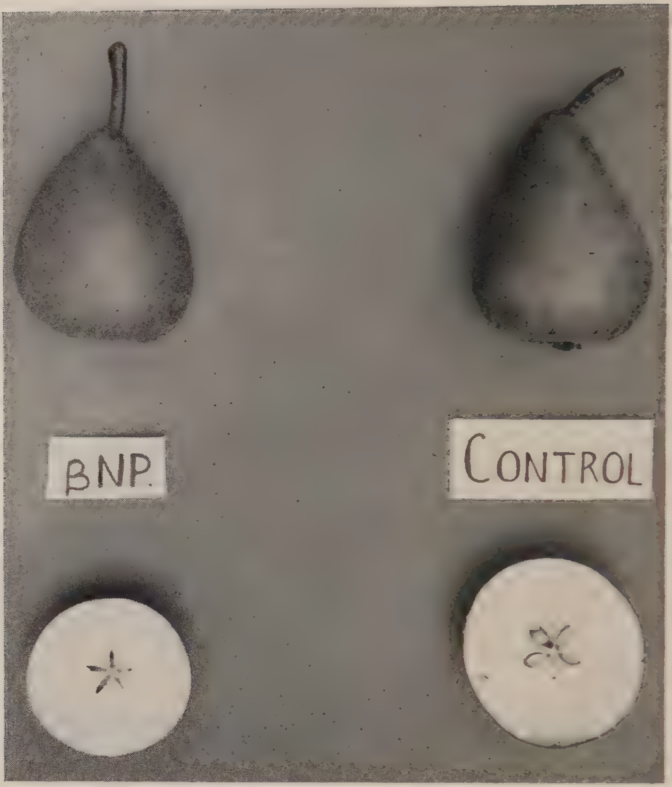

FIG. 3.
Pears var. *Fertility*.
Left : Parthenocarpic pears produced by spraying five times with α-(2-naphthoxy)propionic acid at 100 p.p.m.
Right : Normal seeded pears.

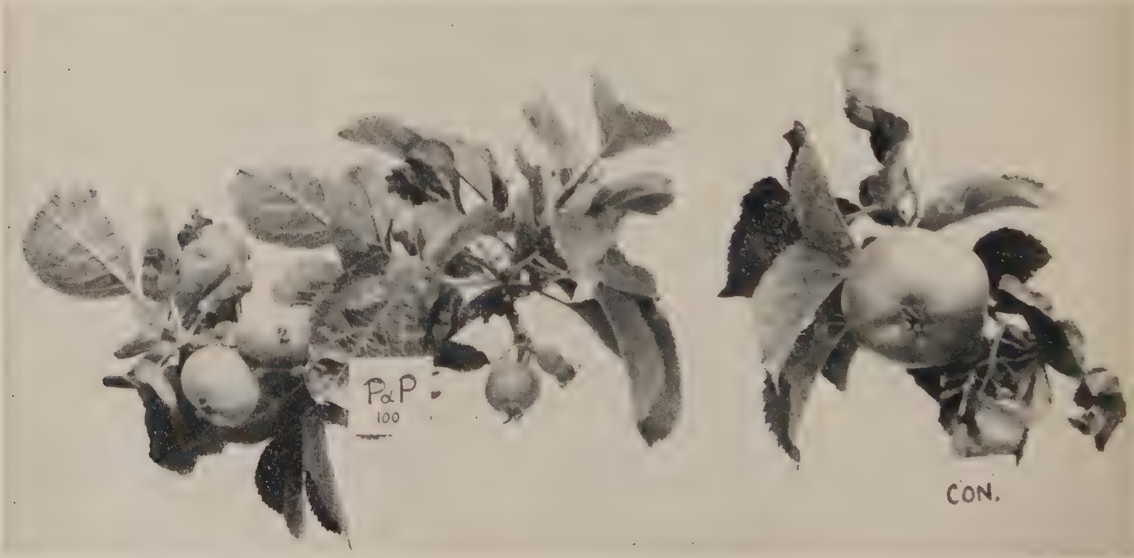

FIG. 4.
Apples var. *Bramley's Seedling*.
Left : Parthenocarpic fruits produced by spraying at three-day intervals with α-phenoxypropionic acid at 100 p.p.m.
Right : Normal seeded fruits.

spray applications. The results presented indicate that for optimum setting of pomaceous fruits an adequate supply of growth substance must be made available during the early stages of development. In the present experiment this was best achieved by applying, at three-day intervals, five single successive sprays containing the compound.

ACKNOWLEDGMENTS.

Thanks are due to Dr. I. W. Selman for his co-operation in the preliminary experiments carried out in 1947 and to Miss R. D. Walker, M.Sc., Dr. M. S. Smith, Mr. R. C. Seeley, B.Sc., and Mr. C. F. Mills, B.Sc., for their help in emasculation of blossoms. The assistance of Miss Walker and Mr. C. H. Fawcett, B.Sc., on the chemical side and of Mr. D. Simons for photographs is also acknowledged.

The work was made possible by the award to one of us (D.J.O.) of a College Fellowship provided by Messrs. May and Baker, Ltd.

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(*Received* 18/10/50.)

REVIEWS

GENTIANS. By David Wilkie. (Country Life Limited, 2-10 Tavistock Street, London, W.C.2. 1950. Pp. 251, 95 illus. Price 25s.)

To those who have not been able to obtain a copy of this book owing to the shortages of the last ten years, Mr. Wilkie's second edition of "Gentians" will be most welcome. First published in 1936, this book, the first of its kind to deal so fully with the genus *Gentiana*, was soon out of print. All those interested in these beautiful but often difficult plants will wish to possess their own copy, as it is a reference work dealing alphabetically with all the gentians in cultivation rather than a book to settle down with in an armchair for an evening's reading.

As Mr. Wilkie states in his introduction, this is not a monograph of the genus, but he has fully described all the gentians known to be grown at the present time as well as those which have been grown in the past and have since been lost.

The main part of the book consists of a full description of each plant in horticultural rather than botanical terms, but in every case reference is made to the original botanical description. The native habitat of each is stated and in most cases the date of introduction as a garden plant, the season of flowering and the most suitable soil and position in the garden. In addition, the reader is given a clear indication as to whether the plant is suitable for the ordinary gardener or would be of interest only to the connoisseur. Species of gentians are found in nearly every country of the world, therefore it is not surprising that general conditions for successful cultivation cannot be outlined and that it is not easy to grow a large number of species in one garden with equal success. One particularly useful chapter groups the commonly grown species according to the place they should occupy in the garden —such as the scree, the shadier parts of the rock garden, the woodland, the border or even the rose garden. The many gentians which are intolerant of lime are noted and gentian lovers who garden at the foot of the chalk downs will welcome the notes on those species which will grow with them. One chapter explains fully the methods of propagation which the writer has found satisfactory and the reader is reminded of the fact that a wide variation of colours may be obtained in plants from one seed capsule, particularly with *G. Farreri*, and that once a good form is obtained this should be propagated by vegetative means. In this second edition several plants which were listed only in the first publication are now fully described and several more recent hybrids are discussed. Mr. Wilkie draws attention to the fact that fifteen plants formerly classified as Crawfurdias are now included in the genus *Gentiana* and are described as such. The new edition contains two hundred and fifty-one pages of text and ninety-five plates which are either photographs of living plants or of herbarium material.

In a genus consisting of more than eight hundred species and many hybrids it would be impossible to describe each of these fully in one volume; Chapter VII consists of an alphabetical list of the species not fully described elsewhere in the book and included in the list are notes on the size and colour of the flower, size of the plant as well as the country of origin. This chapter will be a valuable guide to the traveller who may find many gentians not in cultivation. Students of historical works on horticulture will be interested in the list of names which have been used in the past for *Gentiana*, and of even greater value to the horticulturist is the list of synonyms which have been and still are used for many of the species. How often has the nurseryman been blamed for supplying wrongly named plants!

A welcome addition to the second edition would have been a key to the classification of the genus. At present the grower of gentians who has no access to botanical records must read through pages of descriptions in order to identify his particular specimen. Also gentian lovers may regret that, except for the frontispiece which is a reproduction of a painting of *Gentiana sino-ornata* by John Nash, none of the plates in the book is in colour, especially when so many publications at the present time are illustrated with excellent coloured plates. Nevertheless, Mr. Wilkie's book is a comprehensive and careful survey written with the sure touch of the specialist and gives a wealth of information in a very readable form.

M.P.

AN INTRODUCTION TO PLANT LIFE. By R. P. FAULKNER. (London: W. H. & L. Collingridge, Ltd. 1950. Pp. 120, figs. 26. Price 6s. net.)

This is a small book intended to help the horticulturist who has little or no botanical knowledge, to understand the living plant. In the eleven chapters there are discussed in a lively manner the origin, nutrition, structure, physiology, chemistry, metabolism, reproduction, heredity, seasonal life and, lastly, the plant's enemies.

The book is well produced. A few misprints are noticeable (e.g. pp. 67 and 71), and grammatical errors also occur (e.g. pp. 44, 45, 81).

Great care has been taken to introduce the reader to well established facts of plant life, but, here and there, because exceptions are not mentioned, generalizations take on the appearance of the whole truth. Thus, for example (p. 118), insect transmission of viruses is stated dogmatically to be the only method of spread. Similarly, the prevention of aphid and other insect attack is described as the only means to combat the viruses of tomatoes, dahlias and all other virus-susceptible plants.

An outstanding quality of this work, possibly not appreciated until the whole has been read, is the ease with which the author makes the student familiar with the complicated processes of life and in such a way that the beginner finds himself understanding metabolism, chromosomes, osmosis and the rest without being conscious that he has been made to learn. This is a simple, comprehensive and useful introduction which will appeal to a great many readers, not only to those who are engaged in horticulture.

W.M.W.

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